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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/11868 <b>(22) International Filing Date:</b> 8 June 1998 (08.06.98) <b>(30) Priority Data:</b> 08/880,042 20 June 1997 (20.06.97) US <b>(71) Applicant:</b> UNIVERSITY OF WYOMING [US/US]; Laramie, WY 82070 (US). <b>(72) Inventor:</b> BULLA, Lee, A.; P.O. Box 1629, Laramie, WY 82070 (US). <b>(74) Agents:</b> CIOTTI, Thomas, E. et al.; Morrison & Foerster LLP, 2000 Pennsylvania Avenue, N.W., Washington, DC 20006-1888 (US).	<b>(81) Designated States:</b> AL, AM, AU, AZ, BA, BB, BG, BR, CA, CN, CU, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN  <b>(57) Abstract</b>  The cDNA that encodes a glycoprotein receptor from the tobacco hornworm which binds a <i>Bacillus thuringiensis</i> toxin has been obtained and sequenced. The availability of this cDNA permits the retrieval of DNAs encoding homologous receptors in other insects and organisms as well as the design of assays for the cytotoxicity and binding affinity of potential pesticides and the development of methods to manipulate natural and/or introduced homologous receptors and, thus, to destroy target cells, tissues and/or organisms.		

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RECEPTOR FOR A BACILLUS THURINGIENSIS TOXINAcknowledgment of Government Support

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government has certain rights in this invention.

Technical Field

The invention relates to receptors that bind toxins from *Bacillus thuringiensis*  
and thus to pesticides and pest resistance. More particularly, the invention concerns  
recombinantly produced receptors that bind BT toxin and to their use in assays for  
15 improved pesticides, as well as in mediation of cell and tissue destruction,  
dissociation, dispersion, cell-to-cell association, and changes in morphology.

Background Art

It has long been recognized that the bacterium *Bacillus thuringiensis* (BT)  
20 produces bactericidal proteins that are toxic to a limited range of insects, mostly in the  
orders Lepidoptera, Coleoptera and Diptera. Advantage has been taken of these  
toxins in controlling pests, mostly by applying bacteria to plants or transforming  
plants themselves so that they generate the toxins by virtue of their transgenic  
character. The toxins themselves are glycoprotein products of the *cry* gene as  
25 described by Höfte, H. *et al. Microbiol Rev* (1989) 53:242. It has been established  
that the toxins function in the brush border of the insect midgut epithelial cells as  
described by Gill, S.S. *et al. Annu Rev Entomol* (1992) 37:615. Specific binding of  
BT toxins to midgut brush border membrane vesicles has been reported by Hofmann,  
C. *et al. Proc Natl Acad Sci USA* (1988) 85:7844; Van Rie, J. *et al. Eur J Biochem*  
30 (1989) 186:239; and Van Rie, J. *et al. Appl Environ Microbiol* (1990) 56:1378.

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Presumably, the toxins generated by BT exert their effects by some kind of interaction with receptors in the midgut. The purification of a particular receptor from *Manduca sexta* was reported by the present inventors in an article by Vadlamudi, R.K. *et al. J Biol Chem* (1993) 268:12334. In this report, the receptor protein was isolated by immunoprecipitating toxin-binding protein complexes with toxin-specific antisera and separating the complexes by SDS-PAGE followed by electroelution. However, to date, there has been no structural information concerning any insect receptor which binds BT toxin, nor have, to applicants' knowledge, any genes encoding these receptors been recovered.

#### Disclosure of the Invention

The present invention is based, in part, on the isolation and characterization of a receptor that is bound by members of the BT-toxin family of insecticidal proteins, hereinafter the BT-R<sub>1</sub> protein. The present invention is further based on the isolation and characterization of a nucleic acid molecule that encodes the BT-toxin receptor, hereinafter *BT-R<sub>1</sub>* gene. Based on these observations, the present invention provides compositions and methods for use in identifying agents that bind to the BT-R<sub>1</sub> protein as a means for identifying insecticidal agent and for identifying other members of the BT-R<sub>1</sub> family of proteins.

#### Brief Description of the Drawings

Figure 1 show the nucleotide sequence and deduced amino acid sequence of cDNA encoding the BT-R<sub>1</sub> protein from *M. sexta*.

Figure 2 (panels a and b) shows a comparison of amino acid sequences of cadherin motifs (BTRcad-1 to 11) in BT-R<sub>1</sub> to those of other cadherins.

Figure 3 shows a block diagram of the cadherin-like structure of BT-R<sub>1</sub>.

Figure 4 shows the clone characterization of the BamHI-SacI fragment of BT-R<sub>1</sub>. LM is HindIII cut Lambda marker; UP is the uncut plasmid clone; NP is NsiI cut plasmid; XP is XhoI cut plasmid; BSP is BamHI and SacI cut plasmid showing

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the cloned fragment from BT-R<sub>1</sub>; RM is mRNA size marker; and RT1 and RT2 are transcribed mRNAs from the cloned BT-R<sub>1</sub> fragment.

Figure 5 illustrates the detection of protein expression from the plasmid containing the Bam-Sac fragment of BT-R<sub>1</sub> using <sup>35</sup>S-methionine as a tag. LCR is a  
5 luciferase control mRNA to show that the rabbit reticulocyte lysates are functional; RR1 and RR2 are expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in rabbit reticulocytes from mRNA; LCT is a luciferase control plasmid to show that the transcription/translation kit is functional; and TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in a transcription/translation kit.

10 Figure 6 shows a radio-blot of the Bam-Sac fragment of BT-R<sub>1</sub> with <sup>125</sup>I-labeled Cry1Ab. BBMV is the brush border membrane vesicles from the midgut of *M. sexta* containing the wild-type BT-R<sub>1</sub> receptor protein; RBK is a rabbit reticulocyte blank; RR1 and RR2 are the expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in rabbit reticulocytes from mRNA; TBK is a  
15 transcription/translation kit blank; TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in a transcription/translation kit. The arrows point to two of the bands.

Figure 7 shows the presence of a BT-R<sub>1</sub> homologue in Pink Bollworm and European Corn Borer identified using toxin binding similar to that used to identify the  
20 original BT-R<sub>1</sub> clone.

Figure 8 shows the binding of Cry1Ab to fragments of the BT-R<sub>1</sub> protein.

### Modes of Carrying Out the Invention

#### **I. General Description**

25 The present invention is based, in part, on the isolation and characterization of a novel protein expressed in the midgut of *Manduca sexta* that binds to members of the BT-toxin family of proteins, hereinafter the BT-R<sub>1</sub> protein. The present invention specifically provides purified BT-R<sub>1</sub>, the amino acid sequence of BT-R<sub>1</sub>, as well as nucleotide sequences that encode BT-R<sub>1</sub>. The BT-R<sub>1</sub> protein and nucleic acid  
30 molecules can serve as targets in identifying insecticidal agents.

## II. Specific Embodiments

### A. BT-R<sub>1</sub> Protein

5 Prior to the present invention, although members of the BT-toxin family of protein were known, no one had identified the receptor that is bound by these toxin proteins. The present invention provides, in part, the amino acid sequences of a BT-toxin receptor that is expressed in the midgut of *Manduca sexta*.

10 In one embodiment, the present invention provides the ability to isolate or produce a previously unknown protein by using known purification methods, the cloned nucleic acid molecules herein described or by synthesizing a protein having the amino acid sequence herein disclosed.

As used herein, BT-R<sub>1</sub> refers to a protein that has the amino acid sequence of BT-R<sub>1</sub> provided in Figure 1, as well as allelic variants of the BT-R<sub>1</sub> sequence, and conservative substitutions mutants of the BT-R<sub>1</sub> sequence that have BT-R<sub>1</sub> activity.  
15 BT-R<sub>1</sub> is comprised of a single subunit, has a molecular weight of 210 kD, and has the amino acid sequence provided in Figure 1. A prediction of the structure of BT-R<sub>1</sub> is provided in Figure 3.

The BT-R<sub>1</sub> protein of the present invention includes the specifically identified and characterized variant herein described, as well as allelic variants, conservative  
20 substitution variants and homologues (Figure 7) that can be isolated/generated and characterized without undue experimentation following the methods outlined below. For the sake of convenience, all BT-R<sub>1</sub> proteins will be collectively referred to as the BT-R<sub>1</sub> proteins, the BT-R<sub>1</sub> proteins of the present invention or BT-R<sub>1</sub>.

The term "BT-R<sub>1</sub>" includes all naturally occurring allelic variants of the  
25 *Manduca sexta* BT-R<sub>1</sub> protein provided in Figure 1. In general, naturally occurring allelic variants of *Manduca sexta* BT-R<sub>1</sub> will share significant homology, at least 75 %, and generally at least 90%, to the BT-R<sub>1</sub> amino acid sequence provided in Seq. ID No:2. Allelic variants, though possessing a slightly different amino acid sequence than Seq. ID No:2, will be expressed as a transmembrane protein in the digestive tract  
30 of an insect or other organism. Typically, allelic variants of the BT-R<sub>1</sub> protein will

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contain conservative amino acid substitutions from the BT-R<sub>1</sub> sequence herein described or will contain a substitution of an amino acid from a corresponding position in a BT-R<sub>1</sub> homologue (a BT-R<sub>1</sub> protein isolated from an organism other than *Manduca sexta*).

5           One class of BT-R<sub>1</sub> allelic variants will be proteins that share a high degree of homology with at least a small region of the amino acid sequence provided in Seq. ID No: \_\_, but may further contain a radical departure from the sequence, such as a non-conservative substitution, truncation, insertion or frame shift. Such alleles are termed mutant alleles of BT-R<sub>1</sub> and represent proteins that typically do not perform the same  
10           biological functions as does the BT-R<sub>1</sub> variant of Seq. ID No:2.

          The BT-R<sub>1</sub> proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the BT-R<sub>1</sub> protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard  
15           purification methods to obtain an isolated BT-R<sub>1</sub> protein. The nature and degree of isolation will depend on the intended use.

          The cloning of the BT-R<sub>1</sub> encoding nucleic acid molecule makes it possible to generate defined fragments of the BT-R<sub>1</sub> proteins of the present invention. As discussed below, fragments of BT-R<sub>1</sub> are particularly useful in: generating domain  
20           specific antibodies; identifying agents that bind to toxin binding domain on BT-R<sub>1</sub>; identifying toxin-binding structures; identifying cellular factors that bind to BT-R<sub>1</sub>; isolating homologues or other allelic forms of BT-R<sub>1</sub>; and studying the mode of action of BT-toxins.

          Fragments of the BT-R<sub>1</sub> proteins can be generated using standard peptide  
25           synthesis technology and the amino acid sequence of *Manduca sexta* BT-R<sub>1</sub> disclosed herein. Alternatively, as illustrated in Example 5, recombinant methods can be used to generate nucleic acid molecules that encode a fragment of the BT-R<sub>1</sub> protein. Fragments of the BT-R<sub>1</sub> protein subunits that contain particularly interesting structures can be identified using art-known methods such as by using an  
30           immunogenicity plot, Chou-Fasman plot, Garnier-Robson plot, Kyte-Doolittle plot,



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Eisenberg plot, Karplus-Schultz plot or Jameson-Wolf plot of the BT-R<sub>1</sub> protein. Fragments containing such residues are particularly useful in generating domain specific anti-BT-R<sub>1</sub> antibodies or in identifying cellular factors that bind to BT-R<sub>1</sub>. One particular fragment that is preferred for use in identifying insecticidal agents is a soluble fragment of BT-R<sub>1</sub> that can bind to a member of the BT family of toxins. In Example 5, a fragment of BT-R<sub>1</sub> that binds to a BT-toxin is disclosed.

As described below, members of the BT-R<sub>1</sub> family of proteins can be used for, but are not limited to: 1) a target to identify agents that bind to BT-R<sub>1</sub>, 2) a target or bait to identify and isolate binding partners and cellular factors that bind to BT-R<sub>1</sub>, 3) an assay target to identify BT-R<sub>1</sub> and other receptor-mediated activity, and 4) a marker of cells that express a member of the BT-R<sub>1</sub> family of proteins.

#### **B. Anti-BT-R<sub>1</sub> Antibodies**

The present invention further provides antibodies that bind BT-R<sub>1</sub>. The most preferred antibodies will selectively bind to BT-R<sub>1</sub> and will not bind (or will only bind weakly) to non-BT-R<sub>1</sub> proteins. Anti-BT-R<sub>1</sub> antibodies that are especially contemplated include monoclonal and polyclonal antibodies as well as fragments containing the antigen binding domain and/or one or more complement determining regions (CDRs) of these antibodies.

Antibodies are generally prepared by immunizing a suitable mammalian host using a BT-R<sub>1</sub> protein (synthetic or isolated), or fragment, in isolated or immunoconjugated form (Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). Regions of the BT-R<sub>1</sub> protein that show immunogenic structure can readily be identified using art-known methods. Other important regions and domains can readily be identified using protein analytical and comparative methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Fragments containing these residues are particularly suited in generating specific classes of anti-BT-R<sub>1</sub> antibodies. Particularly useful fragments include, but are not limited to, the BT-toxin binding domain of BT-R<sub>1</sub> identified in Example 5.

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Methods for preparing a protein for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation with reagents such as carbodiimide may be used; in other instances linking reagents like those supplied by Pierce Chemical Co., Rockford, IL, may be effective.

Administration of a BT-R<sub>1</sub> immunogen is conducted generally by injection over a suitable time period in combination with a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

Although the polyclonal antisera produced in this way may be satisfactory for some applications, for many other applications, monoclonal antibody preparations are preferred. Immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the BT-R<sub>1</sub> protein or BT-R<sub>1</sub> fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of F(ab')<sub>2</sub> fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the BT-R<sub>1</sub> protein can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin.

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As described below, anti-BT-R<sub>1</sub> antibodies are useful as modulators of BT-R<sub>1</sub> activity, are useful in *in vitro* and *in vivo* antibody based assays methods for detecting BT-R<sub>1</sub> expression/activity, in generating toxin conjugates, for purifying homologues of *Manduca sexta* BT-R<sub>1</sub>, in generating anti-ideotypic antibodies that mimic the BT-R<sub>1</sub> protein and in identifying competitive inhibitors of BT-toxin/BT-R<sub>1</sub> interactions.

### C. BT-R<sub>1</sub> Encoding Nucleic Acid Molecules

As described above, the present invention is based, in part, on isolating nucleic acid molecules from *Manduca sexta* that encode BT-R<sub>1</sub>. Accordingly, the present invention further provides nucleic acid molecules that encode the BT-R<sub>1</sub> protein, as herein defined, preferably in isolated form. For convenience, all BT-R<sub>1</sub> encoding nucleic acid molecules will be referred to as BT-R<sub>1</sub> encoding nucleic acid molecules, the *BT-R<sub>1</sub>* genes, or *BT-R<sub>1</sub>*. The nucleotide sequence of the *Manduca sexta* nucleic acid molecule that encodes one allelic form of BT-R<sub>1</sub> is provided in Figure 1.

As used herein, a "nucleic acid molecule" is defined as an RNA or DNA molecule that encodes a peptide as defined above, or is complementary to a nucleic acid sequence encoding such peptides. Particularly preferred nucleic acid molecules will have a nucleotide sequence identical to or complementary to the *Manduca sexta* DNA sequences herein disclosed. Specifically contemplated are genomic DNA, cDNAs, synthetically prepared DNAs, and antisense molecules, as well as nucleic acids based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. A skilled artisan can readily obtain these classes of nucleic acid molecules using the herein described *BT-R<sub>1</sub>* sequences. However, such nucleic acid molecules, are defined further as being novel and unobvious over any prior art nucleic acid molecules encoding non-BT-R<sub>1</sub> proteins. For example, the *BT-R<sub>1</sub>* sequences of the present invention specifically excludes previously identified nucleic acid molecules that share only partial homology to *BT-R<sub>1</sub>*. Such excluded sequences include identified members of the cadherin family of proteins.

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As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules that encode polypeptides other than BT-R<sub>1</sub>. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated BT-R<sub>1</sub> encoding nucleic acid molecule.

The present invention further provides fragments of the BT-R<sub>1</sub> encoding nucleic acid molecules of the present invention. As used herein, a fragment of a BT-R<sub>1</sub> encoding nucleic acid molecule refers to a small portion of the entire *BT-R<sub>1</sub>* sequence. The size of the fragment will be determined by its intended use. For example, if the fragment is chosen so as to encode the toxin binding domain of BT-R<sub>1</sub> identified in Example 5, then the fragment will need to be large enough to encode the toxin binding domain of the BT-R<sub>1</sub> protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming. Fragments of the *Manduca sexta BT-R<sub>1</sub>* gene that are particularly useful as selective hybridization probes or PCR primers can be readily identified from the entire *BT-R<sub>1</sub>* sequence using art-known methods.

Another class of fragments of BT-R<sub>1</sub> encoding nucleic acid molecules are the expression control sequence found upstream and downstream from the BT-R<sub>1</sub> encoding region found in genomic clones of the *BT-R<sub>1</sub>* gene. Specifically, tissue and developmental specific expression control elements can be identified as being 5' to the BT-R<sub>1</sub> encoding region found in genomic clones of the *BT-R<sub>1</sub>* gene. Such expression control sequence are useful in generating expression vectors for expressing genes in the digestive tract of a transgenic organism. As described in more detail below, a skilled artisan can readily use the *BT-R<sub>1</sub>* cDNA sequence herein described to isolate and identify genomic *BT-R<sub>1</sub>* sequences and the expression control elements found in the *BT-R<sub>1</sub>* gene.

Fragments of the BT-R<sub>1</sub> encoding nucleic acid molecules of the present invention (i.e., synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding BT-R<sub>1</sub> proteins, can easily be synthesized by chemical techniques, for example, the

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phosphotriester method of Matteucci, *et al.*, *J Am Chem Soc* (1981) 103:3185-3191, or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the *BT-R<sub>1</sub>* gene, followed by ligation of oligonucleotides to build the complete modified *BT-R<sub>1</sub>* gene.

The *BT-R<sub>1</sub>* encoding nucleic acid molecules of the present invention may further be modified so as to contain a detectable label for diagnostic and probe purposes. As described above, such probes can be used to identify nucleic acid molecules encoding other allelic variants or homologues of the *BT-R<sub>1</sub>* proteins and as described below, such probes can be used to identify the presence of a *BT-R<sub>1</sub>* protein as a means for identifying cells that express a *BT-R<sub>1</sub>* protein. A variety of such labels are known in the art and can readily be employed with the *BT-R<sub>1</sub>* encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides, biotin, and the like. A skilled artisan can employ any of the art-known labels to obtain a labeled *BT-R<sub>1</sub>* encoding nucleic acid molecule.

#### D. Isolation of Other *BT-R<sub>1</sub>* Encoding Nucleic Acid Molecules

The identification of the *BT-R<sub>1</sub>* protein from *Manduca sexta* and the corresponding encoding nucleic acid molecules, has made possible the identification of and isolation of: 1) *BT-R<sub>1</sub>* proteins from organisms other than *Manduca sexta*, hereinafter referred to collectively as *BT-R<sub>1</sub>* homologues, 2) other allelic and mutant forms of the *Manduca sexta* *BT-R<sub>1</sub>* protein (described above), and 3) the corresponding genomic DNA that contains the *BT-R<sub>1</sub>* gene. The most preferred source of *BT-R<sub>1</sub>* homologues are insects, the most preferred being members of the Lepidopteran, Coleopteran and Dipteran orders of insects. Evidence of the existence of *BT-R<sub>1</sub>* homologues is provided in Figure 7.

Essentially, a skilled artisan can readily use the amino acid sequence of the *Manduca sexta* *BT-R<sub>1</sub>* protein to generate antibody probes to screen expression libraries prepared from cells and organisms. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described above) or

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monoclonal antibodies can be used to probe an expression library, prepared from a target organism, to obtain the appropriate coding sequence for a BT-R<sub>1</sub> homologue. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructing an expression cassette using  
5 control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the BT-R<sub>1</sub> encoding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the BT-R<sub>1</sub> family of proteins from organisms other than *Manduca sexta*, allelic variants of the *Manduca sexta* BT-R<sub>1</sub> protein herein described, and genomic sequence containing the  
10 *BT-R<sub>1</sub>* gene. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a  
15 polymerase chain reaction (PCR) to selectively amplify/clone a BT-R<sub>1</sub>-encoding nucleic acid molecule, or fragment thereof. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other BT-R<sub>1</sub> encoding nucleic acid molecules. Regions of the *Manduca sexta* *BT-R<sub>1</sub>* gene that are particularly well suited for use as a probe or as primers can be  
20 readily identified by one skilled in the art.

Non-*Manduca sexta* homologues of *BT-R<sub>1</sub>*, naturally occurring allelic variants of the *Manduca sexta* *BT-R<sub>1</sub>* gene and genomic *BT-R<sub>1</sub>* sequences will share a high degree of homology to the *Manduca sexta* *BT-R<sub>1</sub>* sequence herein described. In general, such nucleic acid molecules will hybridize to the *Manduca sexta* *BT-R<sub>1</sub>*  
25 sequence under high stringency. Such sequences will typically contain at least 70% homology, preferably at least 80%, most preferably at least 90% homology to the *Manduca sexta* *BT-R<sub>1</sub>* sequence of Seq. ID No:1.

In general, nucleic acid molecules that encode homologues of the *Manduca sexta* BT-R<sub>1</sub> protein will hybridize to the *Manduca sexta* *BT-R<sub>1</sub>* sequence under  
30 stringent conditions. "Stringent conditions" are those that (1) employ low ionic

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strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium titrate/0.1% SDS at 50°C., or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate  
5 buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2 x SSC and 0.1% SDS. A skilled artisan can  
10 readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

The presence of similar receptors in noninsect organisms as well as other insects besides those harboring BT-R<sub>1</sub> is supported by the sequence similarity of the BT-R<sub>1</sub> protein to that of the various members of the cadherin superfamily of proteins,  
15 which are membrane glycoproteins believed to mediate calcium-dependent cell aggregation and sorting. See, for example, Takeichi, M. *Science* (1991) 251:1451; and Takeichi, M. *N Rev Biochem* (1990) 59:237.

Included in this superfamily are desmoglein, desmocollins, the *Drosophila fat* tumor suppressor, *Manduca sexta* intestinal peptide transport protein and T-cadherin.  
20 All of these proteins share common extracellular motifs although their cytoplasmic domains differ. Goodwin, L. *et al. Biochem Biophys Res Commun* (1990) 173:1224; Holton, J.L. *et al. J Cell Sci* (1990) 97:239; Bestal, D.J. *J Cell Biol* (1992) 119:451; Mahoney, P.A. *et al. Cell* (1991) 853; Dantzig, A.H. *et al. Science* (1994) 264:430; and Sano, K. *et al. EMBO J* (1993) 12:2249. Inclusion of BT-R<sub>1</sub> in the cadherin  
25 superfamily is further supported by the report that EDTA decreases the binding of *CryIAb* toxin of BT to the 210 kD receptor of *M. sexta* (Martinez-Ramirez, A.C. *et al. Biochem Biophys Res Commun* (1994) 201:782).

It is noted below that the amino acid sequence of BT-R<sub>1</sub> reveals that a calcium-binding motif is present. This is consistent with the possibility that cells having  
30 receptors to bind toxin may themselves survive although they render the tissues in

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which they are included permeable to solutes and thus effect disintegration of the tissue. Such a mechanism is proposed for the death of insects that ingest the toxin via the epithelial cells in their midgut by Knowles, B.H. *et al. Biochim Biophys Acta* (1987) 924:509. Such a mechanism is also supported in part by the results set forth in Example 4 hereinbelow which indicate that the effect of the toxin on embryonic 293 cells modified to express the receptor at their surface is reversible.

#### **E. rDNA Molecules Containing a BT-R<sub>1</sub> Encoding Nucleic Acid Molecule**

The present invention further provides recombinant DNA molecules (rDNAs) that contain a BT-R<sub>1</sub> encoding sequences as herein described, or a fragment thereof, such as a soluble fragment of BT-R<sub>1</sub> that contains the BT-toxin binding site. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in vitro*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al., Molecular Cloning* (1989). In the preferred rDNA molecules of the present invention, a BT-R<sub>1</sub> encoding DNA sequence that encodes a BT-R<sub>1</sub> protein or a fragment of BT-R<sub>1</sub>, is operably linked to one or more expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which the BT-R<sub>1</sub> encoding sequence is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of the BT-R<sub>1</sub> encoding sequence included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, enhancers, transcription terminators and other regulatory elements. Preferably, an inducible promoter that is readily controlled, such as being responsive to a nutrient in the host cell's medium, is used. Further, for soluble fragments, it may be desirable to use secretion signals to direct the secretion of the BT-R<sub>1</sub> protein, or fragment, out of the cell.



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In one embodiment, the vector containing a BT-R<sub>i</sub> encoding nucleic acid molecule will include a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule intrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or viral promoter capable of directing the expression (transcription and translation) of the BT-R<sub>i</sub> encoding sequence in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, NJ.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to variant rDNA molecules that contain a BT-R<sub>i</sub> encoding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are PSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (*neo*) gene. Southern *et al.*, *J Mol Anal Genet* (1982)

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1:327-341. Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by cotransfection of the host cell, and selected by culturing in the presence of the appropriate drug for the selectable marker.

5                   **F. Host Cells Containing an Exogenously Supplied BT-R<sub>1</sub> Encoding Nucleic Acid Molecule**

The present invention further provides host cells transformed with a nucleic acid molecule that encodes a BT-R<sub>1</sub> protein of the present invention, either the entire BT-R<sub>1</sub> protein or a fragment thereof. The host cell can be either prokaryotic or eukaryotic.

10 Eukaryotic cells useful for expression of a BT-R<sub>1</sub> protein are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of a *BT-R<sub>1</sub>* gene. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, the most preferred being cells that do not naturally express a BT-R<sub>1</sub> protein.

15                   Any prokaryotic host can be used to express a BT-R<sub>1</sub>-encoding rDNA molecule. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with an rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic

20 host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen *et al.*, *Proc Acad Sci USA* (1972) 69:2110; and Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically

25 employed, see, for example, Graham *et al.*, *Virol* (1973) 52:456; Wigler *et al.*, *Proc Natl Acad Sci USA* (1979) 76:1373-76.

Successfully transformed cells, i.e., cells that contain an rDNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to

30 produce single colonies. Cells from those colonies can be harvested, lysed and their

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DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J Mol Biol* (1975) 98:503, or Berent *et al.*, *Biotech* (1985) 3:208 or the proteins produced from the cell assayed via an immunological method.

5                   **G. Production of a BT-R<sub>1</sub> Protein Using an rDNA Molecule**

The present invention further provides methods for producing a BT-R<sub>1</sub> protein that uses one of the BT-R<sub>1</sub> encoding nucleic acid molecules herein described. In general terms, the production of a recombinant BT-R<sub>1</sub> protein typically involves the following steps.

10                   First, a nucleic acid molecule is obtained that encodes a BT-R<sub>1</sub> protein or a fragment thereof, such as the nucleic acid molecule depicted in Figure 1. The BT-R<sub>1</sub> encoding nucleic acid molecule is then preferably placed in an operable linkage with suitable control sequences, as described above, to generate an expression unit containing the BT-R<sub>1</sub> encoding sequence. The expression unit is used to transform a suitable host  
15                   and the transformed host is cultured under conditions that allow the production of the BT-R<sub>1</sub> protein. Optionally the BT-R<sub>1</sub> protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the  
20                   desired coding sequences may be obtained from genomic fragments and used directly in an appropriate host. The construction of expression vectors that are operable in a variety of hosts is accomplished using an appropriate combination of replicons and control sequences. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail  
25                   earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with BT-R<sub>1</sub> encoding sequences to produce a BT-R<sub>1</sub> protein.

#### **H. Identification of Agents and Cellular Constituents that Bind to a BT-R<sub>1</sub> Protein**

Another embodiment of the present invention provides methods for identifying agents and cellular constituents that bind to BT-R<sub>1</sub>. Specifically, agents and cellular constituents that bind to BT-R<sub>1</sub> can be identified by: 1) the ability of the agent/constituent to bind to BT-R<sub>1</sub>, 2) the ability to block BT-toxin binding to BT-R<sub>1</sub>, and/or 3) the ability to kill BT-R<sub>1</sub> expressing cells. Activity assays for BT-R<sub>1</sub> activity and binding and competitive assays using a BT-R<sub>1</sub> protein are suitable for use in high through-put screening methods, particularly using a soluble fragment of BT-R<sub>1</sub> that contains the BT-toxin binding domain, such as that disclosed in Example 5.

In detail, in one embodiment, BT-R<sub>1</sub> is mixed with an agent or cellular extract. After mixing under conditions that allow association of BT-R<sub>1</sub> with the agent or component of the extract, the mixture is analyzed to determine if the agent/component bound to the BT-R<sub>1</sub>. Binding agents/components are identified as being able to bind to BT-R<sub>1</sub>. Alternatively or consecutively, BT-R<sub>1</sub> activity can be directly assessed as a means for identifying agonists and antagonists of BT-R<sub>1</sub> activity.

Alternatively, targets that are bound by a BT-R<sub>1</sub> protein can be identified using a yeast two-hybrid system or using a binding-capture assay. In the yeast two hybrid system, an expression unit encoding a fusion protein made up of one subunit of a two subunit transcription factor and the BT-R<sub>1</sub> protein is introduced and expressed in a yeast cell. The cell is further modified to contain 1) an expression unit encoding a detectable marker whose expression requires the two subunit transcription factor for expression and 2) an expression unit that encodes a fusion protein made up of the second subunit of the transcription factor and a cloned segment of DNA. If the cloned segment of DNA encodes a protein that binds to the BT-R<sub>1</sub> protein, the expression results in the interaction of the BT-R<sub>1</sub> and the encoded protein. This brings the two subunits of the transcription factor into binding proximity, allowing reconstitution of the transcription factor. This results in the expression of the detectable marker. The yeast two hybrid system is particularly useful in screening a library of cDNA encoding segments for cellular binding partners of BT-R<sub>1</sub>.

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The BT-R<sub>1</sub> protein used in the above assays can be: an isolated and fully characterized protein, a fragment of a BT-R<sub>1</sub> protein (such as a soluble fragment containing the BT-toxin binding site), a cell that has been altered to express a BT-R<sub>1</sub> protein/fragment or a fraction of a cell that has been altered to express a BT-R<sub>1</sub> protein/fragment. Further, the BT-R<sub>1</sub> protein can be the entire BT-R<sub>1</sub> protein or a defined fragment of the BT-R<sub>1</sub> protein. It will be apparent to one of ordinary skill in the art that so long as the BT-R<sub>1</sub> protein or fragment can be assayed for agent binding, e.g., by a shift in molecular weight or activity, the present assay can be used.

The method used to identify whether an agent/cellular component binds to a BT-R<sub>1</sub> protein will be based primarily on the nature of the BT-R<sub>1</sub> protein used. For example, a gel retardation assay can be used to determine whether an agent binds to BT-R<sub>1</sub> or a fragment thereof. Alternatively, immunodetection and biochip technologies can be adopted for use with the BT-R<sub>1</sub> protein. A skilled artisan can readily employ numerous art-known techniques for determining whether a particular agent binds to a BT-R<sub>1</sub> protein.

Agents and cellular components can be further, or alternatively, tested for the ability to block the binding of a BT-toxin to a BT-R<sub>1</sub> protein/fragment. Alternatively, antibodies to the BT-toxin binding site or other agents that bind to the BT-toxin binding site on the BT-R<sub>1</sub> protein can be used in place of the BT-toxin.

Agents and cellular components can be further tested for the ability to modulate the activity of a BT-R<sub>1</sub> protein using a cell-free assay system or a cellular assay system. As the activities of the BT-R<sub>1</sub> protein become more defined, functional assays based on the identified activity can be employed.

As used herein, an agent is said to antagonize BT-R<sub>1</sub> activity when the agent reduces BT-R<sub>1</sub> activity. The preferred antagonist will selectively antagonize BT-R<sub>1</sub>, not affecting any other cellular proteins. Further, the preferred antagonist will reduce BT-R<sub>1</sub> activity by more than 50%, more preferably by more than 90%, most preferably eliminating all BT-R<sub>1</sub> activity.

As used herein, an agent is said to agonize BT-R<sub>1</sub> activity when the agent increases BT-R<sub>1</sub> activity. The preferred agonist will selectively agonize BT-R<sub>1</sub>, not

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affecting any other cellular proteins. Further, the preferred antagonist will increase BT-R<sub>1</sub> activity by more than 50%, more preferably by more than 90%, most preferably more than doubling BT-R<sub>1</sub> activity.

Agents that are assayed in the above method can be randomly selected or  
5 rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences of the BT-R<sub>1</sub> protein or BT-toxin. An example of randomly selected agents is the use of a chemical library or a peptide combinatorial library, or a growth broth of an organism or plant extract.

10 As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis that takes into account the sequence of the target site and/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the BT-R<sub>1</sub> protein and BT-toxin. For example, a rationally selected peptide  
15 agent can be a peptide whose amino acid sequence is identical to a fragment of a BT-R<sub>1</sub> protein or BT-toxin.

The agents tested in the methods of the present invention can be, as examples, peptides, small molecules, and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of  
20 the agents used in the present screening method. One class of agents of the present invention are peptide agents whose amino acid sequences are chosen based on the amino acid sequence of the BT-R<sub>1</sub> protein or BT-toxin. Small peptide agents can serve as competitive inhibitors of BT-R<sub>1</sub> protein activity.

Peptide agents can be prepared using standard solid phase (or solution phase)  
25 peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

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Another class of agents of the present invention are antibodies immunoreactive with critical positions of the BT-R<sub>1</sub> protein. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the BT-R<sub>1</sub> protein intended to be targeted by the antibodies. Critical regions particularly include the BT-toxin binding domain identified in Example 5. Such agents can be used in competitive binding studies to identify second generation BT-R<sub>1</sub> binding agents.

The cellular extracts tested in the methods of the present invention can be, as examples, aqueous extracts of cells or tissues, organic extracts of cells or tissues or partially purified cellular fractions. A skilled artisan can readily recognize that there is no limit as to the source of the cellular extract used in the screening method of the present invention. The preferred source for isolating cellular binding partners of BT-R<sub>1</sub> are cells that express BT-R<sub>1</sub> or cells that are in close proximity to BT-R<sub>1</sub> expressing cells.

An outline of one screening method is as follows. Cells are modified by transfection, retroviral infection, electroporation or other known means, to express a BT-R<sub>1</sub> protein and then cultured under conditions wherein the receptor protein is produced and displayed. If desired, the cells are then recovered from the culture for use in the assay, or the culture itself can be used *per se*.

In the assays, the modified cells are contacted with the candidate toxin and the effect on metabolism or morphology is noted in the presence and absence of the candidate. The effect may be cytotoxic -- i.e., the cells may themselves exhibit one of the indices of cell death, such as reduced thymidine uptake, slower increase in optical density of the culture, reduced exclusion of vital dyes (e.g., trypan blue), increased release of viability markers such as chromium and rubidium, and the like. The differential response between the toxin-treated cells and the cells absent the toxin is then noted. The strength of the toxin can be assessed by noting the strength of the response.

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These assays may be conducted directly as described above or competitively with known toxins. For example, one approach might be to measure the diminution in binding of labeled BT cry toxin in the presence and absence of the toxin candidate.

In addition to simply screening candidates, the screen can be used to devise improved forms of toxins which are more specific or less specific to particular classes of insects as desired. The ability to determine binding affinity ( $K_a$  and  $K_d$ ), dissociation and association rates, and cytotoxic effects of a candidate allows quick, accurate and reproducible screening techniques for a large number of toxins and other ligands under identical conditions which was not possible heretofore. Such information will facilitate the selection of the most effective toxins and ligands for any given receptor obtained from any desired host cell.

Competition assays may also employ antibodies that are specifically immunoreactive with the receptor. Such antibodies can be prepared in the conventional manner by administering the purified receptor to a vertebrate animal, monitoring antibody titers and recovering the antisera or the antibody-producing cells for immortalization, to obtain immortalized cells capable of secreting antibodies of the appropriate specificity. Techniques for obtaining immortalized B cells and for screening them for secretion of the desired antibody are now conventional in the art. The resulting monoclonal antibodies may be more effective than the polyclonal antisera as competition reagents; furthermore, the availability of the immortalized cell line secreting the desired antibody assures uniformity of production of the same reagent over time. The information and the structural characteristics of toxins and ligands tested will permit a rational approach to designing more efficient toxins and ligands. Additionally, such assays will lead to a better understanding of the function and the structure/function relationship of both toxin/ligand and BT-R<sub>1</sub> analogs. In turn, this will allow the development of highly effective toxins/ligands. Ligands include natural and modified toxins, antibodies (anti-receptor and anti-idiotypic antibodies which mimic a portion of a toxin that binds to a receptor, and whatever small molecules bind the receptors.

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**I. Uses of Agents that Bind to a BT-R<sub>1</sub> Protein**

As provided in the Background section, BT-R<sub>1</sub> is the target for the insecticidal activity of BT-toxins. Agents that bind a BT-R<sub>1</sub> protein can be used: 1) to kill BT-R<sub>1</sub> expressing cells, 2) to identify agents that block the interaction of a BT-toxin with BT-R<sub>1</sub> and 3) in methods for identifying cells that express BT-R<sub>1</sub>.

The methods employed in using the BT-R<sub>1</sub> binding agents will be based primarily on the nature of the BT-R<sub>1</sub> binding agent and its intended use. For example, a BT-R<sub>1</sub> binding agent can be used to: deliver a conjugated toxin to a BT-R<sub>1</sub> expressing cell; modulate BT-R<sub>1</sub> activity; directly kill BT-R<sub>1</sub> expressing cells; or screen for and identify competitive binding agents. An agent that inhibits the activity of BT-R<sub>1</sub> can be used to directly inhibit the growth of BT-R<sub>1</sub> expressing cells. Further, identified cellular factors that bind to BT-R<sub>1</sub> can, themselves, be used in binding/competitive assays to identify agonist and antagonists of BT-R<sub>1</sub>.

**J. Methods for Identifying the Presence of a BT-R<sub>1</sub> protein or gene**

The present invention further provides methods for identifying cells, tissues or organisms expressing a BT-R<sub>1</sub> protein or a *BT-R<sub>1</sub>* gene. Such methods can be used to diagnose the presence of cells or an organism that expresses a BT-R<sub>1</sub> protein *in vivo* or *in vitro*. The methods of the present invention are particularly useful in the determining the presence of cells that are a target for BT-toxin activity or for identifying susceptibility of an organism to a BT-toxin or BT-toxin-like agent. Specifically, the presence of a BT-R<sub>1</sub> protein can be identified by determining whether a BT-R<sub>1</sub> protein, or nucleic acid encoding a BT-R<sub>1</sub> protein, is expressed in a cell, tissue or organism.

A variety of immunological and molecular genetic techniques can be used to determine if a BT-R<sub>1</sub> protein is expressed/produced in a particular cell or sample. In general, an extract containing nucleic acid molecules or an extract containing proteins is prepared. The extract is then assayed to determine whether a BT-R<sub>1</sub> protein, or a BT-R<sub>1</sub> encoding nucleic acid molecule, is produced in the cell.

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For example, to perform a diagnostic test based on nucleic acid molecules, a suitable nucleic acid sample is obtained and prepared using conventional techniques. DNA can be prepared, for example, simply by boiling a sample in SDS. The extracted nucleic acid can then be subjected to amplification, for example by using the polymerase chain reaction (PCR) according to standard procedures, such as a RT-PCR method, to selectively amplify a BT-R<sub>1</sub> encoding nucleic acid molecule or fragment thereof. The size or presence of a specific amplified fragment (typically following restriction endonuclease digestion) is then determined using gel electrophoresis or the nucleotide sequence of the fragment is determined (for example, see Weber and May *Am J Hum Genet* (1989) 44:388-339; Davies, J. *et al. Nature* (1994) 371:130-136)). The resulting size of the fragment or sequence is then compared to the known BT-R<sub>1</sub> proteins encoding sequences, for example via hybridization probe. Using this method, the presence of a BT-R<sub>1</sub> protein can be identified.

To perform a diagnostic test based on proteins, a suitable protein sample is obtained and prepared using conventional techniques. Protein samples can be prepared, for example, simply by mixing a sample with SDS followed by salt precipitation of a protein fraction. The extracted protein can then be analyzed to determine the presence of a BT-R<sub>1</sub> protein using known methods. For example, the presence of specific sized or charged variants of a protein can be identified using mobility in an electric field. Alternatively, antibodies can be used for detection purposes. A skilled artisan can readily adapt known protein analytical methods to determine if a sample contains a BT-R<sub>1</sub> protein.

Alternatively, BT-R<sub>1</sub> protein or gene expression can also be used in methods to identify agents that decrease the level of expression of a BT-R<sub>1</sub> gene. For example, cells or tissues expressing a BT-R<sub>1</sub> protein can be contacted with a test agent to determine the effects of the agent on BT-R<sub>1</sub> protein/gene expression. Agents that activate BT-R<sub>1</sub> protein/gene expression can be used as an agonist of BT-R<sub>1</sub> activity whereas agents that decrease BT-R<sub>1</sub> protein/gene expression can be used as an antagonist of BT-R<sub>1</sub> activity.

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### K. Methods to Sensitize Cells

The present invention further provides methods of sensitizing cells such that they become susceptible to killing with a BT-toxin, or a BT-toxin analog.

Specifically, host cells transformed to express BT-R<sub>1</sub> receptor, or a homolog of the BT-R<sub>1</sub> receptor, become sensitive to the mode of action of BT-toxins. The binding of a BT-toxin to a BT-R<sub>1</sub> receptor expressed on the surface of the transformed cells results in induction of a cellular death and apoptosis of the cell expressing the BT-R<sub>1</sub> receptor. Accordingly, the BT-R<sub>1</sub> receptor is an appropriate candidate for use in transforming cells in which it is desirable to induce cell death.

There are numerous situations in which it is desirable to introduce the selected gene into a selected population of cells, thus bringing about cell death. One such example is in the therapeutic treatment of cancer cells. In using specifically targeted vectors for delivery of BT-R<sub>1</sub>-encoding DNA molecules into a tumor cell, tumor cells within a patient can be engineered to express a BT-R<sub>1</sub> protein. Such cells then become susceptible to death upon treatment with a BT-toxin. Since BT-toxin is not normally toxic to mammalian cells, this method is particularly applicable to inducing cell death in particular cells in a mammalian host. Other situations where it may be desirable to stimulate cell death in particular cells or cell lines are in the treatment of autoimmune disorders and in the treatment of cells harboring pathogens, such as malaria or HIV agents.

The choice of the actual steps employed to introduce a BT-R<sub>1</sub>-encoding DNA molecule into a cell to render the cells susceptible to treatment with BT-toxin is based primarily on the cell type that is to be altered, the conditions under which the cell type will be altered, and the overall use envisioned. A skilled artisan can readily adapt art-known methods for use with the BT-R<sub>1</sub>-encoding DNA molecule of the present invention.

### L. Animal Models and Gene Therapy

The *BT-R<sub>1</sub>* gene and the BT-R<sub>1</sub> protein can also serve as a target for generating transgenic organisms in which the pattern of BT-R<sub>1</sub> expression has been altered. For

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example, in one application, BT-R<sub>1</sub> deficient insects or insect cells can be generated using standard knock-out procedures to inactivate a *BT-R<sub>1</sub>* gene, or, if such animals are non-viable, inducible *BT-R<sub>1</sub>* antisense molecules can be used to regulate *BT-R<sub>1</sub>* activity/expression. Alternatively, cells or an organism can be altered so as to contain

5 a *Manduca sexta* BT-R<sub>1</sub> encoding nucleic acid molecule or an antisense-BT-R<sub>1</sub> expression unit that directs the expression of a BT-R<sub>1</sub> protein or an antisense molecule in a tissue specific fashion. In such uses, an organism or cells, for example insects or insect cells, is generated in which the expression of a *BT-R<sub>1</sub>* gene is altered by inactivation or activation and/or replaced by a *Manduca sexta BT-R<sub>1</sub>* gene. This can

10 be accomplished using a variety of art-known procedures such as targeted recombination. Once generated, the *BT-R<sub>1</sub>* expression altered cells or organisms can be used to 1) identify biological and pathological processes mediated by the BT-R<sub>1</sub> protein, 2) identify proteins and other genes that interact with the BT-R<sub>1</sub> protein, 3) identify agents that can be exogenously supplied to overcome a BT-R<sub>1</sub> protein

15 deficiency and 4) serve as an appropriate screen for identifying mutations within the *BT-R<sub>1</sub>* gene that increases or decreases activity.

For example, it is possible to generate transgenic insects, such as members of the dipteran order, expressing the *Manduca sexta* minigene encoding BT-R<sub>1</sub> in a tissue specific-fashion and test the effect of over-expression of the protein in tissues and

20 cells that normally do not contain the BT-R<sub>1</sub> protein.

#### **M. Use of Expression Control Elements of the BT-R<sub>1</sub> Gene**

The present invention further provides the expression control sequences found 5' of the of the newly identified *BT-R<sub>1</sub>* gene in a form that can be used in generating

25 expression vectors. Specifically, the *BT-R<sub>1</sub>* expression control elements, such as the BT-R<sub>1</sub> promoter, that can readily be identified as being 5' from the ATG start codon in the *BT-R<sub>1</sub>* gene, can be used to direct the expression of an operably linked protein encoding DNA sequence. Since BT-R<sub>1</sub> expression is mostly tissue-specific, the expression control elements are particularly useful in directing the expression of an

30 introduced transgene in a tissue specific fashion. A skilled artisan can readily use the

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BT-R<sub>1</sub> gene promoter and other regulatory elements to generate expression vectors using methods known in the art.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

### Example 1

#### Purification and Sequence Determination of BT-R<sub>1</sub> Protein

Midguts of *M. sexta* were extracted and the BT-R<sub>1</sub> protein purified according to the method of Vadlamudi, R.K. *et al. J Biol Chem* (1993) 268:1233, referenced above and incorporated herein by reference. The electroeluted band was confirmed to contain BT-R<sub>1</sub> protein by binding to <sup>125</sup>I-*cryIAb* toxin. In gel electrophoresis, the protein bound to toxin had an apparent weight of approximately 210 kD under reducing and nonreducing conditions.

The purified electroeluted BT-R<sub>1</sub> was subjected to cyanogen bromide digestion and the cyanogen bromide fragments separated on a 17% high-resolution tricine SDS-polyacrylamide gel as described by Schagger, H. *et al. Anal Biochem* (1987) 166:368. The separated fragments were transferred to Problott membranes (Applied Biosystems) and five bands were extracted and subjected to microsequencing using standard instrumentation. The amino acid sequences obtained were:

1. (Met)-Leu-Asp-Tyr-Glu-Val-Pro-Glu-Phe-Gln-Ser-Ile-Thr-Ile-Arg-Val-Val-Ala-Thr-Asp-Asn-Asn-Asp-Thr-Arg-His-Val-Gly-Val-Ala;
2. (Met)-X-Glu-Thr-Tyr-Glu-Leu-Ile-Ile-His-Pro-Phe-Asn-Tyr-Tyr-Ala;
3. (Met)-X-X-X-His-Gln-Leu-Pro-Leu-Ala-Gln-Asp-Ile-Lys-Asn-His;
4. (Met)-Phe/Pro-Asn/Ile-Val-Arg/Tyr-Val-Asp-Ile/Gly;
5. (Met)-Asn-Phe-Phe/His-Ser-Val-Asn-Arg/Asp-Glu.

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Example 2Recovery of cDNA

An *M. sexta* cDNA library was constructed from midgut tissue in  $\lambda$ gt10 using the Superscript Choice System according to the manufacturer's instructions (Life Technologies, Inc.). Degenerate oligonucleotide probes were constructed based on the peptide sequences determined in Example 1 using the methods and approach described in Zhang, S. *et al. Gene* (1991) 105:61. Synthetic oligonucleotides corresponding to peptides 1-3 of Example 1 were labeled with  $\alpha^{32}\text{P}$  using polynucleotide kinase and used as probes as described in the standard cloning manual of Maniatis, T. *et al. Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2nd ed. 1989). A clone hybridizing to all three probes identified from 40 positive clones as hybridizing to all three of the probes was plaque-purified from a screen of  $4 \times 10^5$  recombinants and subcloned into pBluescript (Stratagene). It contained an insert of 5571 bp.

Double-stranded cDNA in pBluescript was sequenced in both directions by the dideoxy termination method with Sequanase (USB) according to the manufacturer's instructions. The sequencing showed an open reading frame of 4584 base pairs or 1528 amino acids along with a polyadenylation signal at position 5561. The sequence obtained and the deduced amino acid sequence is shown in Figure 1.

Thus, the deduced protein has a molecular mass of 172 kD and a pI of approximately 4.5. The amino acid sequences of the cyanogen bromide fragments of native receptor match perfectly within the deduced amino acid sequence. The open reading frame begins with an ATG that is flanked by the consensus translation initiation sequence GAGATGG for eucaryotic mRNAs as described by Kozak, M. *Nucleic Acids Res* (1987) 15:8125.

As shown in Figure 1, the deduced amino acid sequence includes a putative signal, shown underlined, preceding the mature N-terminus Asn-Glu-Arg-etc. Eleven repeats (cad1-cad11) are shown in the extracellular region upstream of the membrane domain, shown with the heavy underline, at positions 1406-1427. The end of the 11th

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repeat is shown with an arrowhead. The positions of the five CNBR fragments are also shown under the complete sequence.

Figure 2 compares the BT-R<sub>1</sub> sequence obtained herein with other members of the cadherin family. Like known cadherins, the external domain of BT-R<sub>1</sub> is highly repetitive and contains 11 repeats (cad1-cad11; see Figure 2 A). The other cadherins compared in Figure 2 B are mouse P cadherin (mP EC1); *Drosophila fat* EC18 (fat EC18) and protocadherin (PC42 EC2), and *Manduca sexta* intestinal transporter (HPT-1-EC-1). The eleven repeats of the cadherin motif in BT-R<sub>1</sub> (cad1-cad11) are individually aligned with a single motif sequence from each of the other members of the cadherin family. Conserved residues are boxed. The greatest similarity of BT-R<sub>1</sub> to the cadherins is with the extracellular repeats of the cadherin motif of mouse P-cadherin, *Drosophila fat* tumor suppressor and the protocadherins, although homologies are not high (20-40 homology and 30-60 percent similarity). The conserved repeats of BT-R<sub>1</sub> included AXDXD, DXE, DXNDXXP, one glutamic acid residue and two glycine residues (Figure 2 B). Motifs A/VXDXD, DXNDN are the consensus sequences for calcium binding and two such regions are present in a typical cadherin repeat. In all repeats of BT-R<sub>1</sub>, the sequence DXNDN is preceded by 8 to 14 hydrophobic amino acids. Similar hydrophobic sequences also have been observed in the cadherins. The length of the hydrophobic stretches suggests that these areas are not transmembrane regions but that they represent J-sheet structures commonly present in cadherin-like repeats. BT-R<sub>1</sub> contains a putative cytoplasmic domain of 101 amino acids, smaller than vertebrate cadherin cytoplasmic domains (160 amino acids), and shows no homology to any of the cadherin cytoplasmic domains or to cytoplasmic domains of other proteins to which it has been compared in a current sequence data base.

To confirm that the sequenced clone encoded full-length BT-R<sub>1</sub> protein, total mRNA was prepared from midguts of *M. sexta* subjected to Northern blot by hybridization with the antisense 4.8 kb SacI fragment of the BT-R<sub>1</sub> cDNA clone. The Northern blot analysis was conducted by hybridizing to the antisense probe at 42°C and 50% formamide, 5 X Denhardt's Reagent, 5 X SSCP and 50 µg/ml salmon sperm

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DNA. The filter was then washed two times with 1 X SSC + 0.1% SDS and two times with 0.15 X SSC + 0.1% SDS at 42°C. Each wash was roughly 20 minutes. The filter was then exposed to X-ray film for 24 hours. The 4.8 kb probe hybridized to a single 5.6 kb band.

5           The BT-R<sub>1</sub> clone was translated using rabbit reticulolysate and the resulting translated products were immunoprecipitated with antisera raised against native protein encoded by BT-R<sub>1</sub>. For the *in vitro* translation, pBluescript plasmid containing BT-R<sub>1</sub> cDNA was linearized and transcribed with T<sub>3</sub> polymerase (Pharmacia). The translation was conducted according to manufacturer's instructions  
10           with nuclease-treated rabbit reticulolysate (Life Technologies, Inc.). After one hour of incubation at 30°C, the reaction mixture was combined with an equal volume of SDS buffer or lysed with 50 mM Tris buffer containing 1% NP40 and 250 mM NaCl (pH 8.0) for immunoprecipitation. Preimmune serum was used as a control.  
15           Translation and immunoprecipitation products were electrophoresed on a 7.5% SDS-polyacrylamide gel fixed, treated with Enhance (Dupont NEN), dried and exposed to X-ray film for 12 hours.

          Two protein bands of approximately 172 kD and 150 kD as determined by SDS-PAGE were obtained; it is postulated that the 150 kD translation product was due to initiation of translation from an internal methionine at amino acid 242. This is  
20           consistent with the observations of Kozak, M. *Mol Cell Biol* (1989) 9:5073.

          Thus, both results confirm that a full-length clone was obtained.

### Example 3

#### Recombinant Production and Characteristics of the BT-R<sub>1</sub> Protein

25           The BT-R<sub>1</sub> cDNA clone was subcloned into the mammalian expression vector pcDNA3 (Invitrogen) and the construct transfected into COS-7 cells. Membranes isolated from the COS-7 transfectants were solubilized, electrophoresed and ligand blotted with <sup>125</sup>I-CryIAb toxin. The cells were harvested 60 hours after transfection, washed with phosphate-buffered saline and lysed by freezing in liquid nitrogen. Cell  
30           membranes were prepared by differential centrifugation as described by Elshourbagy,



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N.A. *et al. J Biol Chem* (1993) 266:3873. Control cells were COS-7 cells transfected with pcDNA3.

The cell membranes (10 µg) were separated on 7.5% SDS-PAGE blotted to a nylon membrane and blocked with Tris-buffered saline containing 5% nonfat dry milk powder, 5% glycerol and 1% Tween-20. The nylon membrane was then incubated with <sup>125</sup>I-CryIAb toxin (2 X 10<sup>5</sup> cpm/ml) for two hours with blocking buffer, dried and exposed to X-ray film at -70°C. The labeled toxin bound to a 210 ± 5 kD protein; the 210 kD band was observed only in lanes containing membranes prepared from either *M. sexta* or COS-7 cells transfected with the BT-R<sub>1</sub> cDNA construct containing 4810 bp of cDNA comprising the open reading frame.

The discrepancy between the 210 kD protein expressed and the calculated 172 kD molecular weight is due to glycosylation of the protein; *in vitro* translation of the cDNA clone, as described above, which does not result in glycosylation, does produce the 172 kD protein. To verify this, the COS-7 produced protein was subjected to digestion with N-glycosidase-F by first denaturing the purified protein by boiling in 1% SDS for 5 minutes followed by addition of NP-40 to a final concentration of 1% in the presence of 0.1% SDS, and then incubating the denatured protein in sodium phosphate buffer, pH 8.5 at 37°C with N-glycosidase-F for 10 hours. Controls were incubated under the same conditions without enzyme. Digestion products were separated on a 7.5% SDS-PAGE and stained with Coomassie brilliant blue. This glycosidase treatment reduced the molecular weight of BT-R<sub>1</sub> protein from 210 to 190 kD; this indicates N-glycosylation at some of the 16 consensus N-glycosylation sites in the protein. Treatment of BT-R<sub>1</sub> with O-glycosidase and neuraminidase did not alter the mobility of the protein.

In addition, embryonic 293 cells were transfected with the BT-R<sub>1</sub> cDNA clone in pcDNA3 and incubated with the labeled toxin (0.32 nM) in the presence of increasing concentrations (0 to 10<sup>-6</sup> M) of unlabeled toxin. Nonspecific binding was measured as bound radioactivity in the presence of 1 TM unlabeled toxin. A value for the dissociation constant (K<sub>d</sub>) of 1015 pM was determined by Scatchard analysis; this

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is approximately the same value that was obtained for the natural receptor as described by Vadlamudi, R.K. *et al. J Biol Chem* (1993) (*supra*).

#### Example 4

##### 5        Physiological Effect of BT Toxin on Modified Embryonic 293 Cells

Both unmodified embryonic 293 cells, and 293 cells which have been modified to produce the BT-R<sub>1</sub> receptor as described in Example 3, when cultured *in vitro* form adherent star-shaped clusters. When BT toxin (200 nM) is added to serum-free medium, the clusters round up and release from the plastic surfaces of the culture dish. This effect is also observed under known conditions of cytotoxicity for 293 cells. The foregoing effect is observed only when the cells are cultured in serum-free medium since the toxin binds to serum and would thus be ineffective under conditions where serum is present.

However, in the presence of anti-receptor antisera, this effect of BT toxin is blocked. Also, when serum is added back to a culture of modified E293 cells which has been treated in serum-free conditions with the toxin, the cells revert to their normal star-shaped adherent cluster shapes. This indicates that the effect of the toxin is reversible.

#### Example 5

##### 20        Identification Of A Fragment Of BT-R<sub>1</sub> That Binds To A BT Toxin

To understand some of the properties of BT-R<sub>1</sub>, research has been undertaken to define the location of the BT-R<sub>1</sub>/Cry1Ab protein-protein interaction. The full-length wild-type amino acid sequence of BT-R<sub>1</sub> is provided in Fig. 1 with a block diagram of a possible cadherin-like structure for BT-R<sub>1</sub> shown in Fig 3. In both figures, restriction digest sites from the cDNA are provided relative to the positions at which they would disrupt the amino acid coding sequence.

A small fragment lying between the BamHI and SacI restriction sites of wild-type BT-R<sub>1</sub> was cloned into the vector pCITE (Novagen). This vector contains transcription/translation sequences designed for use in a rabbit reticulocyte lysate

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(RRL) system. The clone has been analyzed by restriction mapping and mRNA expression (Fig. 4). Lane UP shows the uncut plasmid and lanes NP and XP show restriction digests using NsiI and XhoI, respectively. NsiI is used because it has only one restriction site lying within the Bam-Sac fragment and does not cut anywhere within the pCITE vector. The BSP lane shows the restriction digest of the clone using BamHI and SacI. The digest releases the cloned fragment which separates at about 700 base pairs. The RT1 and RT2 lanes show mRNA transcription from the clone after linearization with XhoI. The mRNA separates at the expected 1350 base pairs.

Protein for analysis has been prepared from this clone in two ways. First, an RRL translation kit was employed to produce protein from the mRNA transcription reaction described above. Second, the plasmid was added directly to an RRL based transcription and translation (TNT) coupled kit. Protein production was detected using <sup>35</sup>S-methionine as a tag (Fig. 5). The LCR lane shows production of luciferase protein from mRNA in an RRL kit and the LCT lane is luciferase protein from a plasmid containing the luciferase coding sequence translated in the TNT kit. Both are positive controls to demonstrate that the two translation kits are operational. The major bands for luciferase translation are observed at 66 kDa. The lanes labeled as RR<sub>1</sub> and RR<sub>2</sub> show expression of the polypeptide sequence of the Bam-Sac fragment of BT-R<sub>1</sub> translated from mRNA in the RRL kit. The lanes TT1 and TT2 are translations from the pCITE plasmid containing the Bam-Sac fragment from the TNT kit. All four lanes possess a major band at 30 kDa which is the expected size of the Bam-Sac fragment with the addition of a coded antibody tag called S-tag. S-tag is part of the multicloning site of pCITE.

The clone was then tested for its ability to bind the insecticidal toxin Cry1Ab. Polypeptide translation of the Bam-Sac fragment of BT-R<sub>1</sub> was carried out in duplicate as described above. The only change is that the <sup>35</sup>S-methionine tag was left out of the reaction mixtures to produce non-radiolabeled proteins. The proteins were separated by SDS-PAGE, blotted to nitrocellulose and hybridized with <sup>125</sup>I-labeled Cry1Ab (Fig. 6). BBMV is wild-type BT-R<sub>1</sub> prepared from the midgut brush border

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membrane vesicles (BBMV) of *M. sexta*, and, is used as a positive control. RBK and TBK are RRL and TNT control reactions prepared without mRNA or plasmid present to determine whether proteins endogenous to either kit bind Cry1Ab. R<sub>1</sub> and RR2 are translations from the RRL kit and TT1 and TT2 are from the TNT kit. A single 30-  
5 kDa band appears in each of these lanes. Two are marked by arrows. These bands demonstrate that the Bam-Sac fragment of BT-R<sub>1</sub> is capable of binding Cry1Ab insecticidal toxin.

To further understand the nature of this binding site, a set of truncation mutants of BT-R<sub>1</sub> was prepared through the use of restriction digests. The cDNA was  
10 digested at specific sites to remove increasingly larger portions of the C-terminus. The restriction enzymes used were NsiI, BamHI, NruI, ClaI, XhoI and StuI (Figs. 1 and 3). The procedure involved linearizing the plasmid at each one of these sites and transcribing up to the truncation. The shortened mRNAs then were translated in an RRL kit blotted to nitrocellulose and hybridized with <sup>125</sup>I-labeled Cry1Ab.  
15 Translation of the wild-type BT-R<sub>1</sub> from the cDNA showed binding to a 172-kDa protein band, the expected size of wild-type BT-R<sub>1</sub>. It also shows smaller bands that bind Cry1Ab although the nature of these bands has not been determined. A blank made by preparing an RRL reaction mixture without any mRNA gives several bands below 66 kDa that show some type of binding of Cry1Ab to the reticulocytes. The  
20 specificity of this binding has not been determined. The truncation mutants created by NsiI, BamHI, NruI, ClaI, XhoI and StuI restriction digests did not show any binding to Cry1Ab except in the region where the reticulocytes bind Cry1Ab. This data demonstrates that the removal of the last 100 amino acids from wild type BT-R<sub>1</sub> by NsiI restriction results in the loss of the ability of BT-R<sub>1</sub> to bind Cry1Ab. This  
25 localizes the toxin binding site on the BT-R<sub>1</sub> clone and provides a soluble fragment of the receptor that can be used in toxin and other binding studies.

A clone of a fragment of BT-R<sub>1</sub>, called the Bam-Sac fragment, has been prepared. It was prepared using BamHI and SacI restriction digests (Fig. 1) and cloning of the resulting fragment into a vector called pCITE. The polypeptide  
30 sequence was translated and tested for binding to the insecticidal toxin Cry1Ab

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(Figure 8). The Bam-Sac fragment binds to Cry1Ab, providing first insight into the location of the Cry1Ab binding site within the BT-R<sub>1</sub> sequence. It lies in the last 234 C-terminal amino acids. This evidence is further supported by a set of truncation mutants that has been prepared. Removal of the 100 most C-terminal amino acids from wild type BT-R<sub>1</sub> results in the loss of Cry1Ab binding. The C-terminal end of BT-R<sub>1</sub> is the location of the Cry1Ab binding site.

#### Example 6

##### Identification Of Homologue of BT-R<sub>1</sub> That Binds To A BT Toxin

Western blots of tissue extracts prepared from Pink bollworm and European corn borer were prepared and probed with labeled Cry1a (Figure 7). The results show that homologues of BT-R<sub>1</sub> are present in these two insects and can be readily isolated using the methods described herein.

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Claims

1. A method to identify agents that bind to a BT-toxin receptor, said method comprising the steps of:

- 5           i)       contacting an agent with a BT-toxin binding receptor selected from the group consisting of a) a cell that has been altered to contain a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No:2, b) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of the amino acid sequence of SEQ ID No:2 that binds to a BT toxin, c) a cell that has been altered to contain a
- 10       nucleic acid molecule encoding a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, d) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency and that binds to a BT toxin, e) an isolated protein with an amino acid sequence of SEQ
- 15       ID No:2, f) an isolated fragment of a protein with an amino acid sequence of SEQ ID No:2, said fragment containing a BT-toxin binding domain, g) an isolated BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and h) an isolated fragment of a BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the
- 20       nucleic acid sequence of SEQ ID No:1 under high stringency, and
- ii)       determining whether said agent binds to said BT-toxin receptor.

2. The method of claim 1, wherein said method further comprises the step of determining whether said agent blocks the binding of a BT-toxin to said BT-toxin

25       receptor.

3. The method of claim 1, wherein said cell that has been altered is a eukaryotic cell.

30           4. The method of claim 3, wherein eukaryotic cell is an insect cell.

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5. A method to identify agents that block the binding of a BT-toxin to a BT-toxin receptor, said method comprising the steps of:

- 5 i) contacting an agent, in the presence and absence of a BT-toxin, with a BT-toxin binding receptor selected from the group consisting of a) a cell that has been altered to contain a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No:2, b) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of the amino acid sequence of SEQ ID No:2 that binds to a BT toxin, c) a cell that has been altered to contain a nucleic acid molecule encoding a  
10 BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, d) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency and that binds to a BT toxin, e) an isolated protein with an amino acid sequence of SEQ ID No:2, f) an isolated fragment of a  
15 protein with an amino acid sequence of SEQ ID No:2, said fragment containing a BT-toxin binding domain, g) an isolated BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and h) an isolated fragment of a BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of  
20 SEQ ID No:1 under high stringency, and
- ii) determining whether said agent blocks the binding of said BT-toxin to said BT-toxin receptor.

6. The method of claim 5, wherein said BT-toxin is a member of the BT-cry(1) family of toxins.  
25

7. The method of claim 5, wherein said cell that has been altered is a eukaryotic cell.

8. The method of claim 7, wherein eukaryotic cell is an insect cell.  
30

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9. An isolated antibody, wherein said antibody binds to a protein selected from the group consisting of a) a BT-toxin receptor protein with an amino acid sequence of SEQ ID No:2, and b) a BT-toxin receptor protein that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, or a fragment of said antibody, wherein said antibody fragment binds to said BT-toxin.

10. The antibody of claim 9, wherein said antibody binds to said BT-toxin receptor and blocks the binding of a BT-toxin to said receptor.

11. The antibody of claim 10, wherein said antibody binds to an epitope located within the 232 c-terminal amino acids of the BT-toxin receptor depicted in SEQ ID No:2.

12. An isolated BT-toxin receptor protein selected from the group consisting of a) a BT-toxin receptor protein with an amino acid sequence of SEQ ID No:2, b) a BT-toxin receptor protein that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, c) a fragment of a BT-toxin receptor protein with an amino acid sequence of SEQ ID No:2, said fragment being able to bind to a BT-toxin, and d) a fragment of a BT-toxin receptor protein that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, said fragment being able to bind to a BT-toxin.

13. A method to produce BT-toxin receptor protein, or a fragment thereof, said method comprising the steps of:

i) culturing a cell that has been altered to contain a nucleic acid molecule that encodes a BT-toxin receptor protein, or BT-toxin binding fragment thereof, wherein said cell has been altered to contain a nucleic acid molecule selected from the



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group consisting of a) a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No:2, b) a nucleic acid molecule that encodes a fragment of the amino acid sequence of SEQ ID No:2 that binds to a BT toxin, c) a nucleic acid molecule encoding a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and d) a nucleic acid molecule that encodes a fragment of a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency and that binds to a BT toxin, under condition in which said nucleic acid molecule is expressed and

ii) isolating said BT-toxin receptor protein or fragment.

10

14. The method of claim 13, wherein said cell that has been altered is a eukaryotic cell.

15. The method of claim 14, wherein eukaryotic cell is an insect cell.

[illegible]

Fig 1.

CTC ACC ACT CCG CTG CAT ATA TTC ACC GTG ACA GCG CTG GAC TCG CTC CCG AAC ACC CAC ACG GTG ACT ATG ATG GTG CAA GTG GTG AAT  
 Val Thr Ser Pro Leu His Ile Phe Ser Val Thr Ala Leu Asp Ser Leu Pro Asn Thr His Thr Val Thr Met Met Val Gln Val Ala Asn  
 1040 1050 1060 1070 1080 1090 1100 1110 1120  
 GTG AAC ACC CGT CCG CCG CTG TCG CTG GAG ATC TTC GCT GTC CAA CAG TTT GAA CAG AAA TCT TAC CAA AAC TTC ACA GTG AAG GCG AAT  
 Val Asn Ser Arg Pro Pro Arg Trp Leu Glu Ile Phe Ala Val Gln Gln Phe Glu Lys Ser Tyr Gln Asn Phe Thr Val Arg Ala Ile  
 1130 1140 1150 1160 1170 1180 1190 1200 1210  
 GAC GGA GAC ACT GAG ATC AAT ATG CCT ATC AAC TAC AGC CTG ATC ACA AAT GAG GAA CAC ACA TTC TTC AGC ATT GAG GGC CTG CCT GGT  
 Asp Gly Asp Thr Glu Ile Asn Met Pro Ile Asn Tyr Arg Leu Ile Thr Asn Glu Glu Asp Thr Phe Phe Ser Ile Glu Ala Leu Pro Gly  
 1220 1230 1240 1250 1260 1270 1280 1290 1300  
 GGA AAA ACC GGT GCT GTA TTC CTC GTG TCG CCA ATT GAC CCG GAC ACA CTC CAA CCA GAG GTG TTT CCA CTT ACG ATC GTC GCT TAC AAA  
 Gly Lys Ser Gly Ala Val Phe Leu Val Ser Pro Ile Asp Arg Asp Thr Leu Gln Arg Glu Val Phe Pro Leu Thr Ile Val Ala Tyr Lys  
 1310 1320 1330 1340 1350 1360 1370 1380 1390  
 TAT GAT GAG GAG GCC TTC TCC ACA TCA ACA AAC GTG GTC ATC ATT GTC ACA GAC ATC AAC CAC CAA CCA CCT GAA CTT ATA CAC AAG GAA  
 Tyr Asp Glu Glu Ala Phe Ser Thr Ser Thr Asn Val Val Ile Ile Val Thr Asp Ile Asn Asp Gln Arg Pro Glu Pro Ile His Lys Glu  
 1400 1410 1420 1430 1440 1450 1460 1470 1480  
 TAT CGA CTG GCA ATC ATG CAG CAG ACG CCC CTG ACC CTC AAC TTC CAT AAA GAA TTC CCA TTT CAT GAT AAG CAT TTA GGT CAA AAT GAT  
 Tyr Arg Leu Ala Ile Met Glu Glu Thr Pro Leu Thr Leu Asn Phe Asp Lys Glu Phe Gly Phe His Asp Lys Asp Leu Gly Gln Asn Ala  
 1490 1500 1510 1520 1530 1540 1550 1560 1570  
 CAG TAC ACG CTG CCG CTA GAG AGC GTG GAC CCT CCA GGC GCT GCT GAG GCA TTC TAC ATA GCG CCG GAA GTC GCG TAC CAG CTA CAG ACC  
 Gln Tyr Thr Val Arg Leu Glu Ser Val Asp Pro Pro Gly Ala Ala Glu Ala Phe Tyr Ile Ala Pro Glu Val Gly Tyr Gln Arg Gln Thr  
 1580 1590 1600 1610 1620 1630 1640 1650 1660  
 TTC ATC ATG CCG ACC CTC AAT CAC TCC ATG CTG GAT TAC GAA CTG CCA GAG TTT CAG ACT ATT ACG ATT CCG GTG GTA CCG ACC GAC AAC  
 Phe Ile Met Gly Thr Leu Asn His Ser Met Leu Asp Tyr Glu Val Pro Glu Phe Gln Ser Ile Thr Ile Arg Val Val Ala Thr Asp Asn  
 1670 1680 1690 1700 1710 1720 1730 1740 1750  
 AAC GAC ACG ACG CAC GTG CCG CTC CCG TTG GTT CAC ATT GAC CTC ATC AAT TCG AAC GAT CAG CAG CCG ATC TTC CAA CAC GCG GTG CAG  
 Asn Asp Thr Arg His Val Gly Val Ala Leu Val His Ile Asp Leu Ile Asn Trp Asn Asp Glu Gln Pro Ile Phe Glu His Ala Val Gln  
 1760 1770 1780 1790 1800 1810 1820 1830 1840  
 ACC GTC ACC TTC GAC GAG ACT GAA GCG GAG CCG TTC TTC GTC GCC AAG CCG GTT CCA CAC CAC ACA GAC ATC CCG GAT GTC GTC GAG CAT  
 Thr Val Thr Phe Asp Glu Thr Glu Gly Glu Gly Phe Phe Val Ala Lys Ala Val Ala His Asp Arg Asp Ile Gly Asp Val Val Glu His  
 1850 1860 1870 1880 1890 1900 1910 1920 1930  
 ACT TTA TTG CGT AAC CCT GTT AAC TTC CTG ACC ATC GAC AAA CTC ACC GCG GAC ATC CCC CTG TCA GGT AAC GAC TCC TTC AAC TAC CAT  
 Thr Leu Leu Gly Asn Ala Val Asn Phe Leu Thr Ile Asp Lys Leu Thr Gly Asp Ile Arg Val Ser Ala Asn Asp Ser Phe Asn Tyr His  
 1940 1950 1960 1970 1980 1990 2000 2010 2020

10 / 10  
 X 1

CGA GAA AGT GAA TTA TTT CTG CAG GTG CGA GCT ACA GAC ACG CTG GCG GAA CCC TTC CAC ACG GCG ACC TCA CAG CTG CTT ATA CGA CTA  
Arg Glu Ser Glu Leu Phe Val Gln Val Arg Ala Thr Asp Thr Leu Gly Glu Pro Phe His Thr Ala Thr Ser Gln Leu Val Ile Arg Leu  
2030 2040 2050 2060 2070 2080 2090 2100 2110  
AAT GAC ATC AAC AAC ACG CCA ACC ACC TTA CCG CTG CCT CGA GCG AGT CCC CAA CTG CAG GAG AAC GTC CCT GAT GCG GAT GTC ATC ATT  
Asn Asp Ile Asn Asn Thr Pro Thr Leu Arg Leu Pro Arg Gly Ser Pro Gln Val Glu Glu Asn Val Pro Asp Gly His Val Ile Thr  
2120 2130 2140 2150 2160 2170 2180 2190 2200  
CAG GAG TTA CCG GCG ACC GAC CCC GAC ACC ACG GCG GAT CTG CCG TTC CAG ATA AAC TCG GAC ACC TCT TTC GCG ACC AAG CAA GCG GTC  
Gln Glu Leu Arg Ala Thr Asp Pro Asp Thr Thr Ala Asp Leu Arg Phe Glu Ile Asn Trp Asp Thr Ser Phe Ala Thr Lys Gln Gly Arg  
2210 2220 2230 2240 2250 2260 2270 2280 2290  
CAG GCT AAC CCC GAC GAG TTT AGG AAT TCG GTG GAA ATC GAG ACC ATC TTC CCC GAG ATT AAC AAC GCG CGA CTG GAT ATC GCG GAC CTT  
Gln Ala Asn Pro Asp Glu Phe Arg Asn Cys Val Glu Ile Glu Thr Ile Phe Pro Glu Ile Asn Asn Arg Gly Leu Ala Ile Gly Arg Val  
2300 2310 2320 2330 2340 2350 2360 2370 2380  
GTA CCG GCG GAA ATC ACA CAC AAC GTG ACC ATA GAC TAC GAG GAG TTT GAG GTC CTC CTC ACA CTG AGG GTG CCG GAC CTT AAC ATT  
Val Ala Arg Glu Ile Arg His Asn Val Thr Ile Asp Tyr Glu Glu Phe Glu Val Leu Ser Leu Thr Val Arg Val Arg Asp Leu Asn Thr  
2390 2400 2410 2420 2430 2440 2450 2460 2470  
GTC TAC GGA GAC GAC TAC CAG GAA TCG ATC CTC ACA ATA ACT ATA ATC GAT ATG AAC GAG AAC GCG CCG GTG TCG GTG CAG GAA ATT CTT  
Val Tyr Gly Asp Asp Tyr Asp Glu Ser Met Leu Thr Ile Thr Ile Ile Asp Met Asn Asp Asn Ala Pro Val Tip Val Glu Gly Thr Leu  
2480 2490 2500 2510 2520 2530 2540 2550 2560  
GAG CAG AAC TTC CGA CTC CCC GAG ATG TCG CCG GCG GCG CTC GTG GCG CTC GCG GAG GAC ATC GAC GAA CCG CTC CTA AAC  
Glu Gln Asn Phe Arg Val Arg Glu Met Ser Ala Gly Gly Leu Val Val Gly Ser Val Arg Ala Asp Asp Ile Asp Gly Pro Leu Tyr Asn  
2570 2580 2590 2600 2610 2620 2630 2640 2650  
CAA GTG CGA TAC ACC ATT TTC CCT CCT GAA GAC ACA GAT AAG GAC CTG ATA ATG ATC GAC TTC CTC ACG GGT CAA ATT TCC GTG AAC ACA  
Gln Val Arg Tyr Thr Thr Ile Phe Pro Arg Glu Asp Thr Asp Lys Asp Leu Ile Met Ile Arg Phe Leu Thr Gly Ile Ser Val Asp Thr  
2660 2670 2680 2690 2700 2710 2720 2730 2740  
AGC GCG GCG ATC GAC CCG GAT ACT CCT CCT CCA CCG TTC CAC CTC TAC TAT ACA GTG CTC GCT AGT GAC CCA TCG TCG ACA GAA GAT CTT GAA  
Ser Gly Ala Ile Asp Ala Thr Thr Pro Pro Arg Phe His Leu Tyr Tyr Thr Val Val Ala Ser Asp Arg Cys Ser Thr Glu Asp Pro Ala  
2750 2760 2770 2780 2790 2800 2810 2820 2830  
GAT TGC CCC CCT GAC CCG ACT TAT TCG GAA ACC GAA GGA AAT ATC ACA ATC CAC ATC ACC GAG ACC AAC AAC AAG GTC CCG CAG GCG GAA  
Asp Cys Pro Pro Asp Pro Thr Tyr Trp Glu Thr Glu Gly Asn Ile Thr Ile His Ile Thr Asp Thr Asn Asn Lys Val Pro Gln Ala Glu  
2840 2850 2860 2870 2880 2890 2900 2910 2920  
ACG ACT AAG TTC GAT ACC CTC CTG TAT ATT TAC GAG AAC GCA ACC CAC TTA GAG GAG CTC ATT CTG ATA GCG ACT GAT CTT GAT AAT  
Thr Thr Lys Phe Asp Thr Val Val Tyr Ile Tyr Glu Asn Ala Thr His Leu Asp Glu Val Val Thr Leu Ile Ala Ser Asp Leu Asp Arg  
2930 2940 2950 2960 2970 2980 2990 3000 3010

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2930	2940	2950	2960	2970	2980	2990	3000	3010
GAC GAA ATA TAC CAC AGG CTG ACC TAC CTC ATC AAT TAT GCA CTG AAC CCT CGA CTC ATG AAC TTC TTC TCC CTS AAC GCA GAG ACC GAC Asp Glu Ile Tyr His Thr Val Ser Tyr Val Ile Asn Tyr Ala Val Asn Pro Arg Leu Met Asn Phe Phe Ser Val Asn Arg Glu Thr Gly								
3020	3030	3040	3050	3060	3070	3080	3090	3100
CTG GTC TAC GTC GAC TAT GAG ACC CAG GGT AGT GGC GAG CTG CTG GAC CGT GAT GGT GAT GAA CCA AGC CAC CGT ATC TTC TTC AAC CTC Leu Val Tyr Val Asp Tyr Glu Thr Gln Gly Ser Gly Glu Val Leu Asp Arg Asp Gly Asp Glu Pro Thr His Arg Ile Phe Phe Asn Leu								
3110	3120	3130	3140	3150	3160	3170	3180	3190
ATC GAC AAC TTC ATG GGG GAA GCA GAA GGT AAC AGA AAT CAG AAC CAC ACA GAA GTT CTC GTT ATC TTC TTC GAT CTC GAT CAC AAT CCT Ile Asp Asn Phe Met Gly Glu Gly Glu Gln Asn Arg Asn Gln Asn Asp Thr Glu Val Leu Val Ile Leu Leu Asp Val Asn Asp Asn Ala								
3200	3210	3220	3230	3240	3250	3260	3270	3280
CCT GAA TTG CCA CCG CCG ACC GAA CTC TCT TCG ACT ATA TCT GAG AAC CTT AAG CAG GCG CTC CTT CTT GAA CCA CAT ATC TTC GGC CCG Pro Glu Leu Pro Pro Pro Ser Glu Leu Ser Trp Thr Ile Ser Glu Asn Leu Lys Gln Gly Val Arg Leu Glu Pro His Ile Phe Ala Pro								
3290	3300	3310	3320	3330	3340	3350	3360	3370
GAC CCG GAC GAG CCC GAC ACA GAC AAC TCC AGG GTC GGC TAC GAG ATC CTG AAC CTC AGC AGC GAG CAG ATC GAA CTS CCG CAG CTC Asp Arg Asp Glu Pro Asp Thr Asp Asn Ser Arg Val Gly Tyr Glu Ile Leu Asn Leu Ser Thr Glu Arg Asp Ile Glu Val Pro Glu Leu								
3380	3390	3400	3410	3420	3430	3440	3450	3460
TTT GTG ATG ATA CAG ATC CCG AAC CTC ACC GCA GAG CTG CAG ACC GCC ATG GAC CTC AAC GCA TAT TCG GCG ACC TAC OCT ATA GAT ATA Phe Val Met Ile Gln Ile Ala Asn Val Thr Gly Glu Leu Glu Thr Ala Met Asp Leu Lys Gly Tyr Trp Gly Thr Tyr Ala Ile His Ile								
3470	3480	3490	3500	3510	3520	3530	3540	3550
CGG GCA TTC GAC CAC GCG ATT CCG CAA ATG TCC ATG AAC GAG ACA TAT GAG CTG ATC ATC CAT CCG TTC AAC TAC TAC CCG CCT GAG TTC Ala Phe Asp His Gly Ile Pro Gln Met Ser Met Asn Glu Thr Tyr Glu Leu Ile Ile His Pro Phe Asn Tyr Tyr Ala Pro Glu Phe								
3560	3570	3580	3590	3600	3610	3620	3630	3640
GTC TTC CCG ACC AAC GAT GCC CTC ATA CGA CTT CCG ACC GAA CGA GCT GTA ATC AAT GGA GTT CTA CCG ACA GTG AAC GCA GAG TTC TTC Val Phe Pro Thr Asn Asp Ala Val Ile Arg Leu Ala Arg Glu Arg Ala Val Ile Asn Gly Val Leu Ala Thr Val Asn Gly Glu Phe Leu								
3650	3660	3670	3680	3690	3700	3710	3720	3730
CAG CCG ATA TCG GCG ACT GAT CCG GAC GGA CTC CAC GCG GGC GTC CTC ACC TTC CAA GTG GTA GGC CAT GAG GAA TCA CAA CCG TAC TTT Glu Arg Ile Ser Ala Thr Asp Pro Asp Gly Leu His Ala Gly Val Thr Phe Gln Val Val Gly Asp Glu Ser Gln Arg Tyr Phe								
3740	3750	3760	3770	3780	3790	3800	3810	3820
CAA GTA GTT AAC GAT GGC GAG AAC CTC GCG TCG TTG ACC TTA CTC CAA GCC GTT CCA GAG GAG ATC AUG CAG TTC CCG ATA AUG ATT CAG Gln Val Val Asn Asp Gly Glu Asn Leu Gly Ser Leu Arg Leu Leu Gln Ala Val Pro Glu Glu Ile Arg Glu Phe Arg Ile Thr Ile Arg								
3830	3840	3850	3860	3870	3880	3890	3900	3910

100 (100)

Ex. 11. A

**KEY TO UPDATED SEQUENCE:**

unpublished nucleotide number

Type of change

41	'G' addition
2105-00	rearrangement
2627-0	deletion
2660	'C' addition
2678	'G' addition
2946	rearrangement
3464	rearrangement
3471	rearrangement
4035-6	deletion
4084-4892	addition
4938	addition
5030	deletion
5031	rearrangement
5200	rearrangement
5216	rearrangement
5288	deletion
5423	rearrangement

Fig. 1. Co-M.

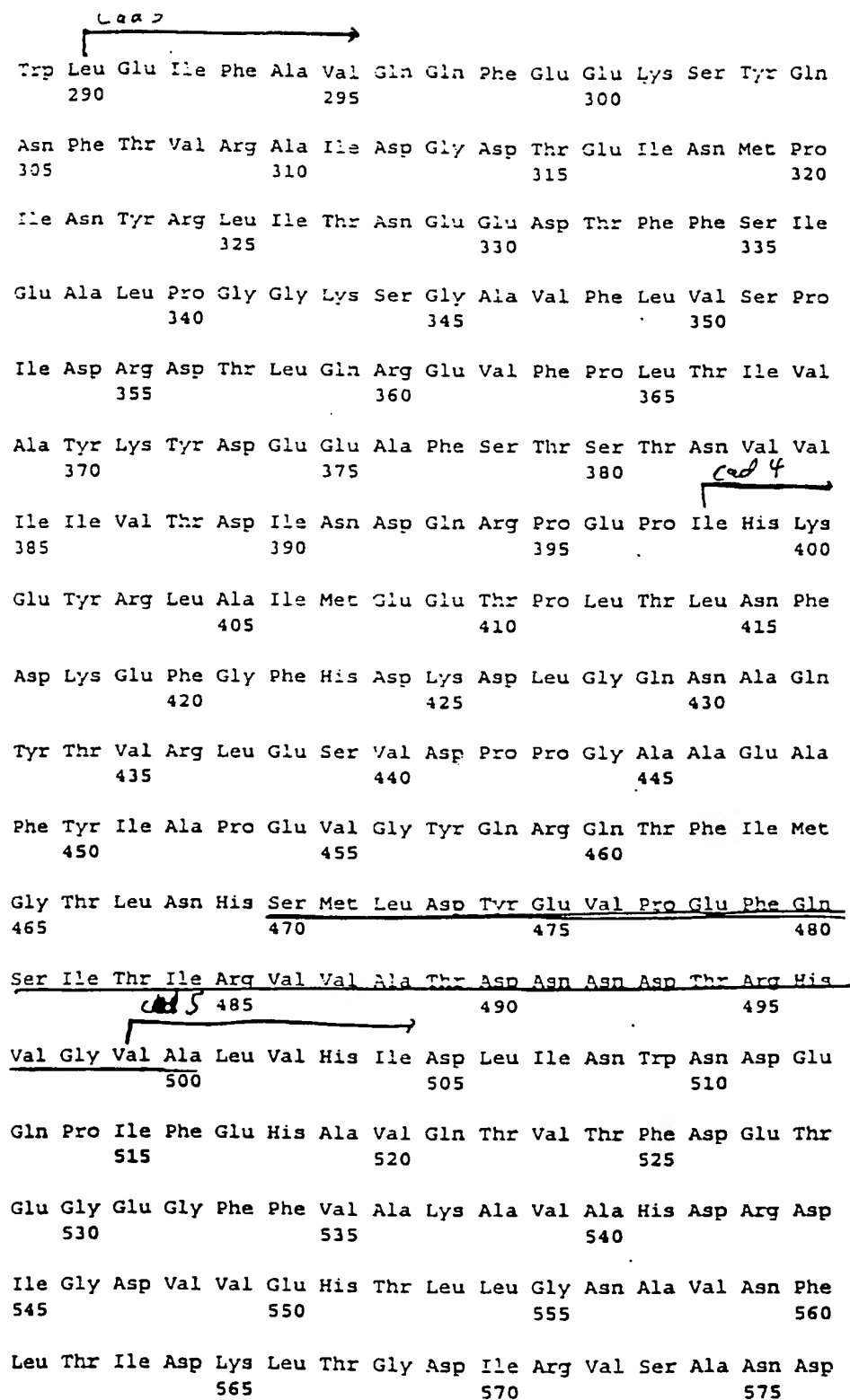


FIGURE 2



Ser Phe Asn Tyr His Arg Glu Ser Glu Leu Phe Val Gln Val Arg Ala  
 580 585 590  
 Thr Asp Thr Leu Gly Glu Pro Phe His Thr Ala Thr Ser Gln Leu Val  
 595 600 605 cad 6  
 Ile Arg Leu Asn Asp Ile Asn Asn Thr Pro Pro Thr Leu Arg Leu Pro  
 610 615 620  
 Arg Gly Ser Pro Gln Val Glu Glu Asn Val Pro Asp Gly His Val Ile  
 625 630 635 640  
 Thr Gln Glu Leu Arg Ala Thr Asp Pro Asp Thr Thr Ala Asp Leu Arg  
 645 650 655  
 Phe Glu Ile Asn Trp Asp Thr Ser Phe Ala Thr Lys Gln Gly Arg Gln  
 660 665 670  
 Ala Asn Pro Asp Glu Phe Arg Asn Cys Val Glu Ile Glu Thr Ile Phe  
 675 680 685  
 Pro Glu Ile Asn Asn Arg Gly Leu Ala Ile Gly Arg Val Val Ala Arg  
 690 695 700  
 Glu Ile Arg His Asn Val Thr Ile Asp Tyr Glu Glu Phe Glu Val Leu  
 705 710 715 720  
 Ser Leu Thr Val Arg Val Arg Asp Leu Asn Thr Val Tyr Gly Asp Asp  
 725 730 735  
 Tyr Asp Glu Ser Met Leu Thr Ile Thr Ile Ile Asp Met Asn Asp Asn  
 740 cad 7 745 750  
 Ala Pro Val Trp Val Glu Gly Thr Leu Glu Gln Asn Phe Arg Val Arg  
 755 760 765  
 Glu Met Ser Ala Gly Gly Leu Val Val Gly Ser Val Arg Ala Asp Asp  
 770 775 780  
 Ile Asp Gly Pro Leu Tyr Asn Gln Val Arg Tyr Thr Ile Phe Pro Arg  
 785 790 795 800  
 Glu Asp Thr Asp Lys Asp Leu Ile Met Ile Asp Phe Leu Thr Gly Gln  
 805 810 815  
 Ile Ser Val Asn Thr Ser Gly Ala Ile Asp Ala Asp Thr Pro Pro Arg  
 820 825 830  
 Phe His Leu Tyr Tyr Thr Val Val Ala Ser Asp Arg Cys Ser Thr Glu  
 835 840 845  
 Asp Pro Ala Asp Cys Pro Pro Asp Pro Thr Tyr Trp Glu Thr Glu Gly  
 850 855 860

FIGURE 2

Asn Ile Thr Ile His Ile Thr Asp Thr Asn Asn Lys Val Pro Gln Ala  
 865 cod 8 870 875 880  
 Glu Thr Thr Lys Phe Asp Thr Val Val Tyr Ile Tyr Glu Asn Ala Thr  
 885 890 895  
 His Leu Asp Glu Val Val Thr Leu Ile Ala Ser Asp Leu Asp Arg Asp  
 900 905 910  
 Glu Ile Tyr His Thr Val Ser Tyr Val Ile Asn Tyr Ala Val Asn Pro  
 915 920 925  
 Arg Leu Met Asn Phe Phe Ser Val Asn Arg Glu Thr Gly Leu Val Tyr  
 930 935 940  
 Val Asp Tyr Glu Thr Gln Gly Ser Gly Glu Val Leu Asp Arg Asp Gly  
 945 950 955 960  
 Asp Glu Pro Thr His Arg Ile Phe Phe Asn Leu Ile Asp Asn Phe Met  
 965 970 975  
 Gly Glu Gly Glu Gly Asn Arg Asn Gln Asn Asp Thr Glu Val Leu Val  
 980 985 cod 990  
 Ile Leu Leu Asp Val Asn Asp Asn Ala Pro Glu Leu Pro Pro Pro Ser  
 995 1000 1005  
 Glu Leu Ser Trp Thr Ile Ser Glu Asn Leu Lys Gln Gly Val Arg Leu  
 1010 1015 1020  
 Glu Pro His Ile Phe Ala Pro Asp Arg Asp Glu Pro Asp Thr Asp Asn  
 1025 1030 1035 1040  
 Ser Arg Val Gly Tyr Glu Ile Leu Asn Leu Ser Thr Glu Arg Asp Ile  
 1045 1050 1055  
 Glu Val Pro Glu Leu Phe Val Met Ile Gln Ile Ala Asn Val Thr Gly  
 1060 1065 1070  
 Glu Leu Glu Thr Ala Met Asp Leu Lys Gly Tyr Trp Gly Thr Tyr Ala  
 1075 1080 1085  
 Ile His Ile Arg Ala Phe Asp His Gly Ile Pro Gln Met Ser Met Asn  
 1090 1095 1100  
 Glu Thr Tyr Glu Leu Ile Ile His Pro Phe Asn Tyr Tyr Ala Pro Glu  
 1105 cod 10 1110 1115 1120  
 Phe Val Phe Pro Thr Asn Asp Ala Val Ile Arg Leu Ala Arg Glu Arg  
 1125 1130 1135  
 Ala Val Ile Asn Gly Val Leu Ala Thr Val Asn Gly Glu Phe Leu Glu  
 1140 1145 1150

FIGURE 2

Arg Ile Ser Ala Thr Asp Pro Asp Gly Leu His Ala Gly Val Val Thr  
 1155 1160 1165  
 Phe Gln Val Val Gly Asp Glu Glu Ser Gln Arg Tyr Phe Gln Val Val  
 1170 1175 1180  
 Asn Asp Gly Glu Asn Leu Gly Ser Leu Arg Leu Leu Gln Ala Val Pro  
 1185 1190 1195 1200  
 Glu Glu Ile Arg Glu Phe Arg Ile Thr Ile Arg Ala Thr Asp Gln Gly  
 1205 1210 1215  
 Thr Asp Pro Gly Pro Leu Ser Thr Asp Met Thr Phe Arg Val Val Phe  
 1220 1225 *cad II* 1230  
 Val Pro Thr Gln Gly Glu Pro Arg Phe Ala Ser Ser Glu His Ala Val  
 1235 1240 1245  
 Ala Phe Ile Glu Lys Ser Ala Gly Met Glu Glu Ser His Gln Leu Pro  
 1250 1255 1260  
 Leu Ala Gln Asp Ile Lys Asn His Leu Cys Glu Asp Asp Cys His Ser  
 1265 1270 1275 1280  
 Ile Tyr Tyr Arg Ile Ile Asp Gly Asn Ser Glu Gly His Phe Gly Leu  
 1285 1290 1295  
 Asp Pro Val Arg Asn Arg Leu Phe Leu Lys Lys Glu Leu Ile Arg Glu  
 1300 1305 1310  
 Gln Ser Ala Ser His Thr Leu Gln Val Ala Ala Ser Asn Ser Pro Asp  
 1315 1320 1325  
 Gly Gly Ile Pro Leu Pro Ala Ser Ile Leu Thr Val Thr Val Thr Val  
 1330 1335 1340  
 Arg Glu Ala Asp Pro Arg Pro Val Phe Val Arg Glu Leu Tyr Thr Ala  
 1345 1350 1355 1360  
 Gly Ile Ser Thr Ala Asp Ser Ile Gly Arg Glu Leu Leu Arg Leu His  
 1365 1370 1375  
 Ala Thr Gln Ser Glu Gly Ser Ala Ile Thr Tyr Ala Ile Asp Tyr Asp  
 1380 1385 1390  
 Thr Met Val Val Asp Pro Ser Leu Glu Ala Val Arg Gln Ser Ala Phe  
 1395 1400 1405  
 Val Leu Asn Ala Gln Thr Gly Val Leu Thr Leu Asn Ile Gln Pro Thr  
 1410 1415 1420  
 Ala Thr Met His Gly Leu Phe Lys Phe Glu Val Thr Ala Thr Asp Thr  
 1425 1430 1435 1440

FIGURE 2

Ala Gly Ala Gln Asp Arg Thr Asp Val Thr Val Tyr Val Val Ser Ser  
1445 1450 1455

Gln Asn Arg Val Tyr Phe Val Phe Val Asn Thr Leu Gln Gln Val Glu  
1460 1465 1470

Asp Asn Arg Asp Phe Ile Ala Asp Thr Phe Ser Ala Gly Phe Asn Met  
1475 1480 1485

Thr Cys Asn Ile Asp Gln Val Val Pro Ala Asn Asp Pro Val Thr Gly  
1490 1495 1500

Val Ala Leu Glu His Ser Thr Gln Met Ala Ala Thr Ser Tyr Gly Thr  
1505 1510 1515 1520

Thr Tyr Pro Tyr Ser Leu Met Arg  
1525

FIGURE 2

Cadherin Consensus Motif ---E...-----G.....A.D.D.....-----

Cadherin Consensus Motif  
G.....DRE.....D.ND..P.F

RECEIVED

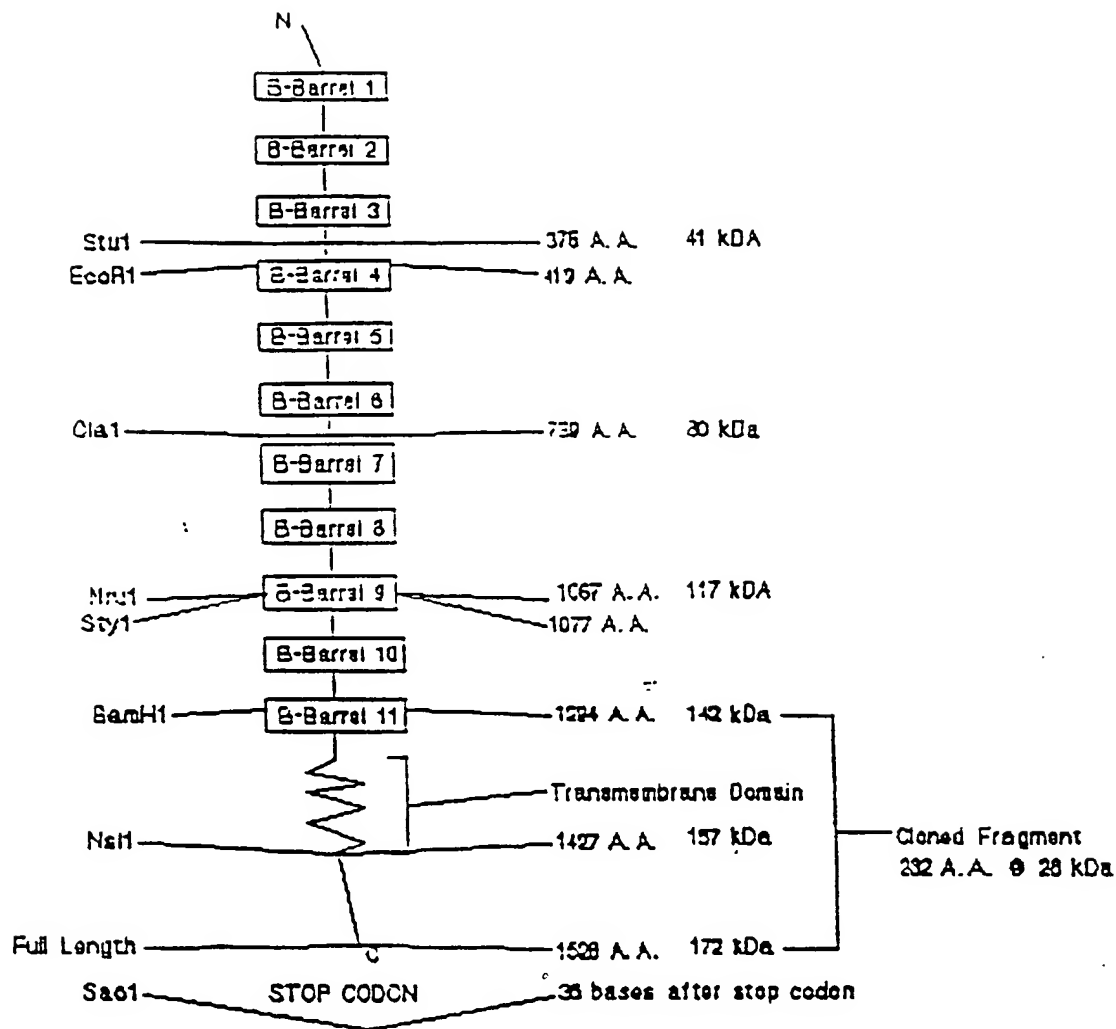
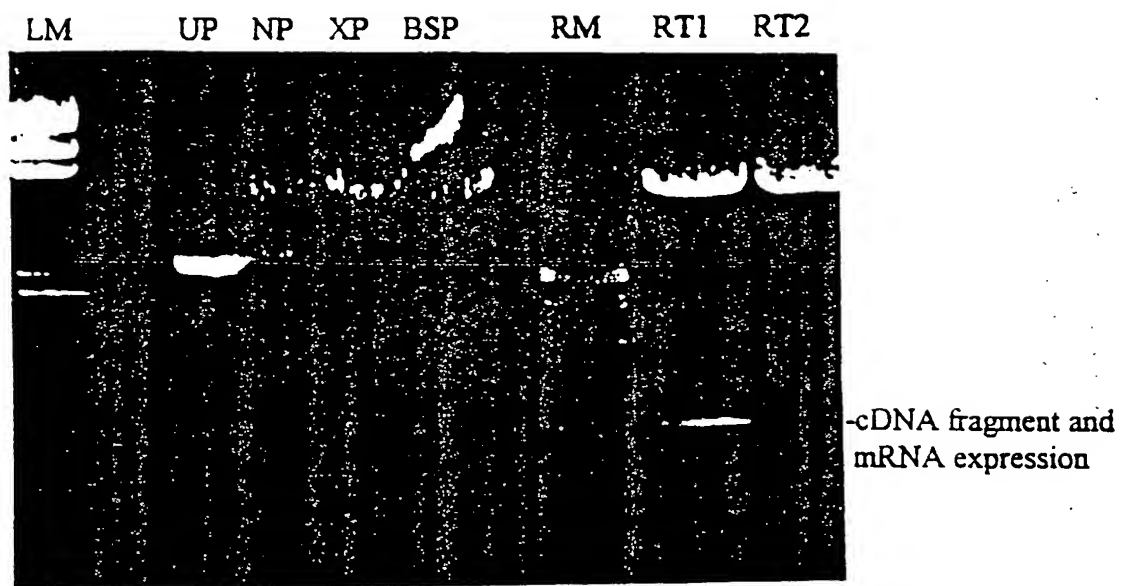
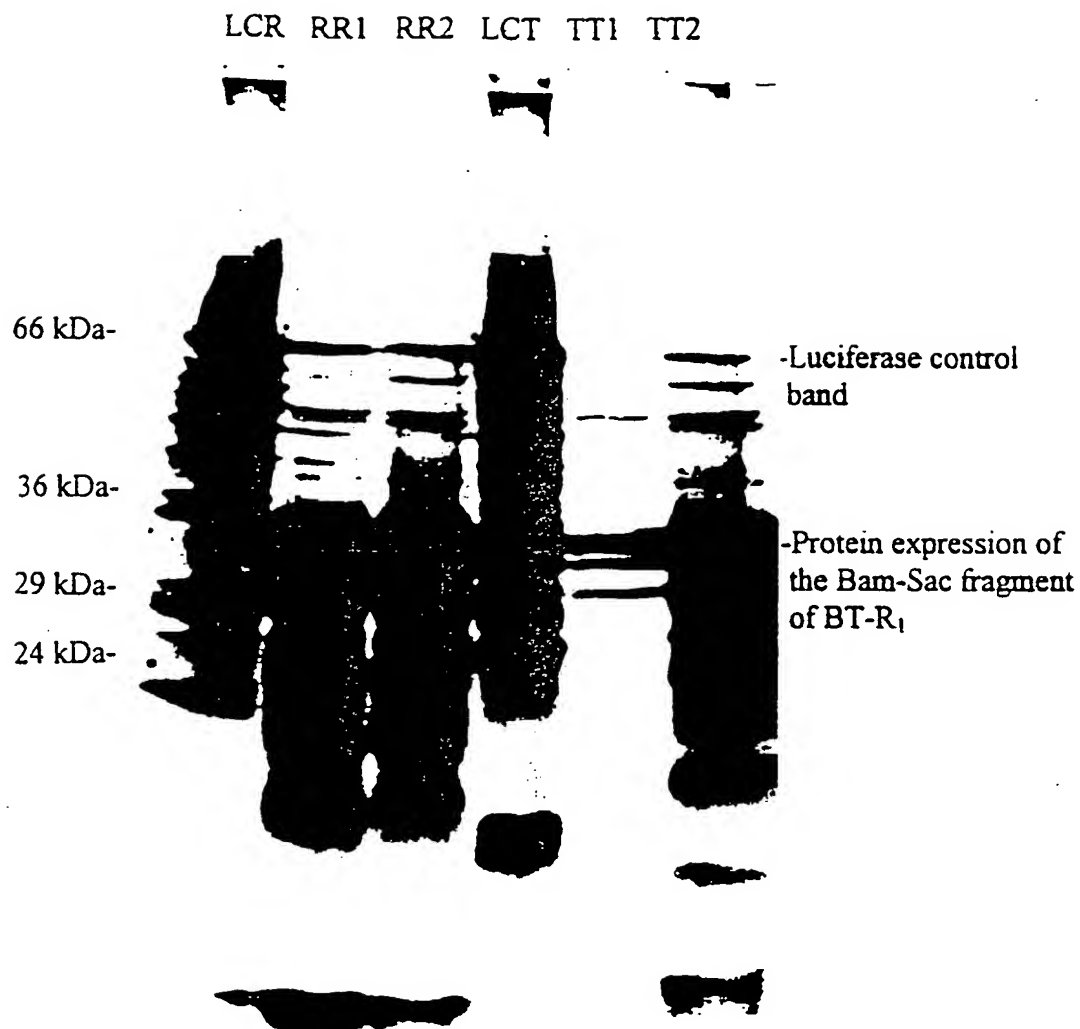


Fig. 3 Block diagram of cadherin-like structure of BT-R<sub>1</sub>

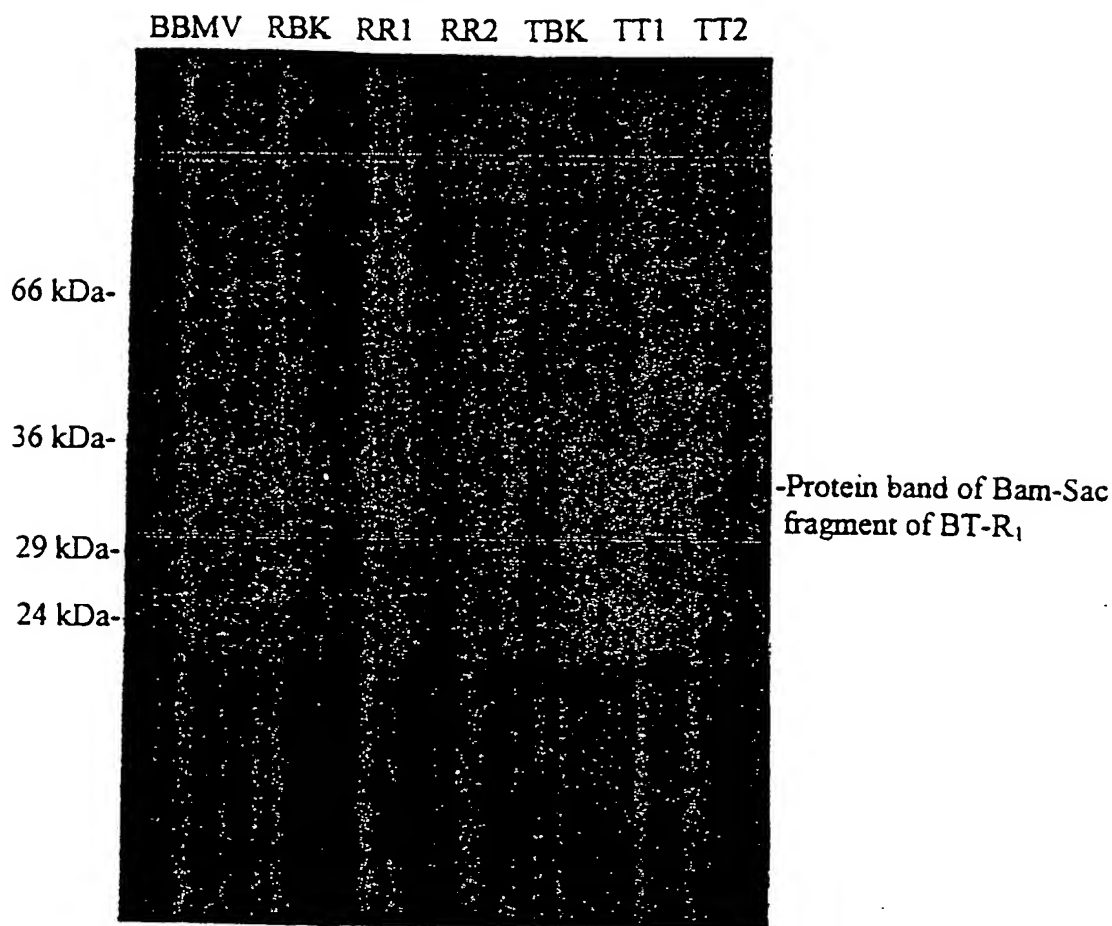


**Fig. 4** Clone characterization of BamHI-SacI fragment of BT-R<sub>1</sub>. LM is HindIII cut Lambda marker; UP is the uncut plasmid clone; NP is NsiI cut plasmid; XP is XhoI cut plasmid; BSP is BamHI and SacI cut plasmid showing the cloned fragment from BT-R<sub>1</sub>; RM is mRNA size marker; and RT1 and RT2 are transcribed mRNAs from the cloned BT-R<sub>1</sub> fragment.



**Fig. 5** Detection of protein expression from the plasmid containing the Bam-Sac fragment of BT-R<sub>1</sub> using <sup>35</sup>S-methionine as a tag. LCR is a luciferase control mRNA to show that the rabbit reticulocyte lysates are functional; RR1 and RR2 are expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in rabbit reticulocytes from mRNA; LCT is a luciferase control plasmid to show that the transcription/translation kit is functional; and TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in a transcription/translation kit.





**Fig. 6** Radio-blot of the Bam-Sac fragment of BT-R<sub>1</sub> with <sup>125</sup>I-labeled Cry1Ab. BBMV is the brush border membrane vesicles from the midgut of *M. Sexta* containing the wild-type BT-R<sub>1</sub> receptor protein; RBK is a rabbit reticulocyte blank; RR1 and RR2 are the expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in rabbit reticulocytes from mRNA; TBK is a transcription/translation kit blank; TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R<sub>1</sub> produced in a transcription/translation kit. The arrows point to two of the bands.

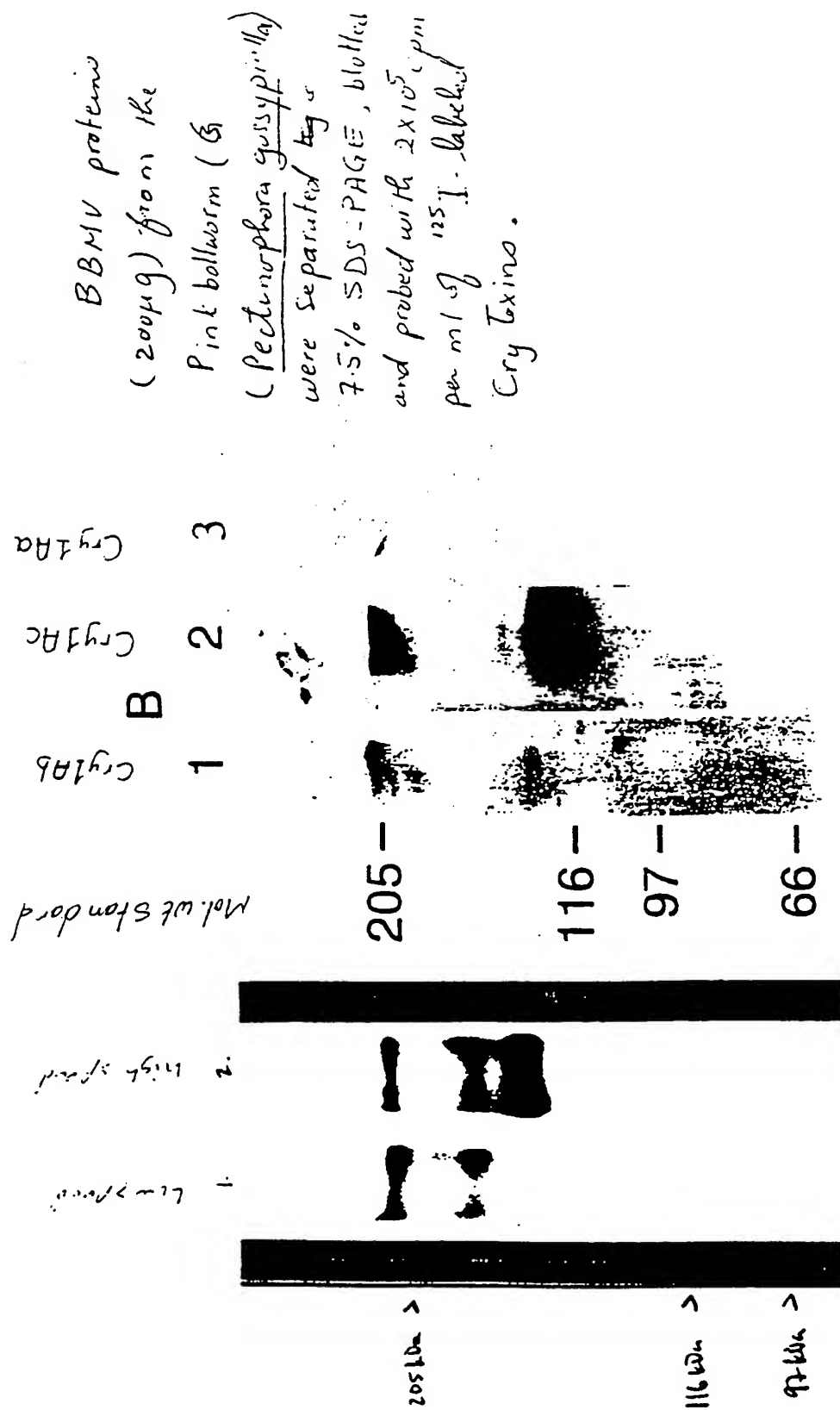


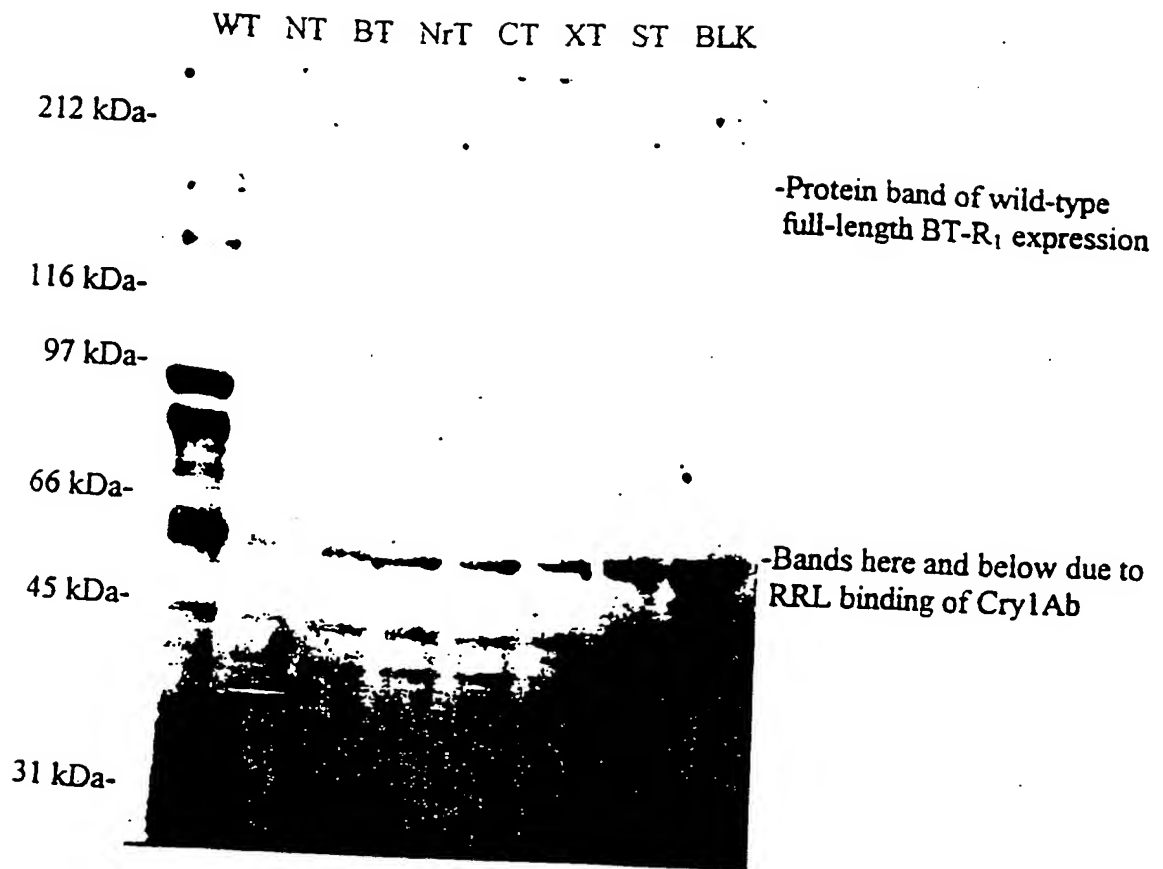
Fig 7

(can be read)

Met Ala Val Asp Val Arg Ile Ala Ala Phe Leu Leu Val Phe Ile Ala  
 1 5 10 15  
 Pro Ala Val Leu Ala Gln Glu Arg Cys Gly Tyr Met Thr Ala Ile Pro  
 20 25 30  
 Arg Leu Pro Arg Pro Asp Asn Leu Pro Val Leu Asn Phe Glu Gly Gln  
 35 40 45  
 Thr Trp Ser Gln Arg Pro Leu Leu Pro Ala Pro Glu Arg Asp Asp Leu  
 50 55 60  
 Cys Met Asp Ala Tyr His Val Ile Thr Ala Asn Leu Gly Thr Gln Val  
 65 70 75 80  
 Ile Tyr Met Asp Glu Glu Ile Glu Asp Glu Ile Thr Ile Ala Ile Leu  
 85 90 95  
 Asn Tyr Asn Gly Pro Ser Thr Pro Phe Ile Glu Leu Pro Phe Leu Ser  
 100 105 110  
 Gly Ser Tyr Asn Leu Leu Met Pro Val Ile Arg Arg Val Asp Asn Gly  
 115 120 125  
 Ser Ala Ser His His His Ala Arg Gln His Tyr Glu Leu Pro Gly Met  
 130 135 140  
 Gln Gln Tyr Met Phe Asn Val Arg Val Asp Gly Gln Ser Leu Val Ala  
 145 150 155 160  
 Gly Val Ser Leu Ala Ile Val Asn Ile Asp Asp Asn Ala Pro Ile Ile  
 165 170 175  
 Gln Asn Phe Glu Pro Cys Arg Val Pro Glu Leu Gly Glu Pro Gly Leu  
 180 185 190  
 Thr Glu Cys Thr Tyr Gln Val Ser Asp Ala Asp Gly Arg Ile Ser Thr  
 195 200 205  
 Glu Phe Met Thr Phe Arg Ile Asp Ser Val Arg Gly Asp Glu Glu Thr  
 210 215 220  
 Phe Tyr Ile Glu Arg Thr Asn Ile Pro Asn Gln Trp Met Trp Leu Asn  
 225 230 235 240  
 Met Thr Ile Gly Val Asn Thr Ser Leu Asn Phe Val Thr Ser Pro Leu  
 245 250 255  
 His Ile Phe Ser Val Thr Ala Leu Asp Ser Leu Pro Asn Thr His Thr  
 260 265 270  
 Val Thr Met Met Val Gln Val Ala Asn Val Asn Ser Arg Pro Pro Arg  
 275 280 285

Cadd1  
 Cadd2

FIGURE 2



**Fig. 8** Radio-blot of BT-R<sub>1</sub> and truncation mutants of BT-R<sub>1</sub> with <sup>125</sup>I-labeled Cry1Ab. WT is the wild-type full-length BT-R<sub>1</sub> receptor; NT is the truncation mutant resulting from NsiI digestion; BT is the mutant made with BamHI; NrT is the mutant made with NruI; CT is the mutant made with ClaI; XT is the mutant made with XhoI; ST is the mutant made with StuI; and, BLK is rabbit reticulocyte lysates containing only endogenous proteins.

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/11868

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/435 C07K16/18 G01N33/56 //C07K14/325

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 12964 A (UNIV WYOMING) 2 May 1996  see page 3, line 9 - line 17 see page 7 - page 8 see claims 1-14	1-10, 12-15
A		11
X	VADLAMUNDI R.K.: "Cloning and expression of a receptor for an insecticidal toxin of <i>Bacillus thuringiensis</i> " J. BIOL. CHEM., vol. 270, no. 10, 10 March 1995, pages 5490-5494, XP002080803 see the whole document	1-10, 12-14

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

14 October 1998

Date of mailing of the international search report

23/10/1998

Name and mailing address of the ISA

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Authorized officer

Galli, I

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11868

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>DORSCH J.A. &amp; BULLA L.A.: "Determination of the specific region of BT-R1 to which the CryaAb toxin of Bacillus thuringiensis subsp. Berliner binds."            FASEB JOURNAL (ABSTRACTS),            vol. 11, no. 9, 31 July 1997, page A1050            XP002080380            see the whole document            -----</p>	11

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/11868

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9612964 A	02-05-1996	US 5693491 A	02-12-1997
		AU 4001595 A	15-05-1996
		CA 2200427 A	02-05-1996
		EP 0787299 A	06-08-1997
		JP 10508198 T	18-08-1998
		ZA 9508851 A	11-06-1996
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